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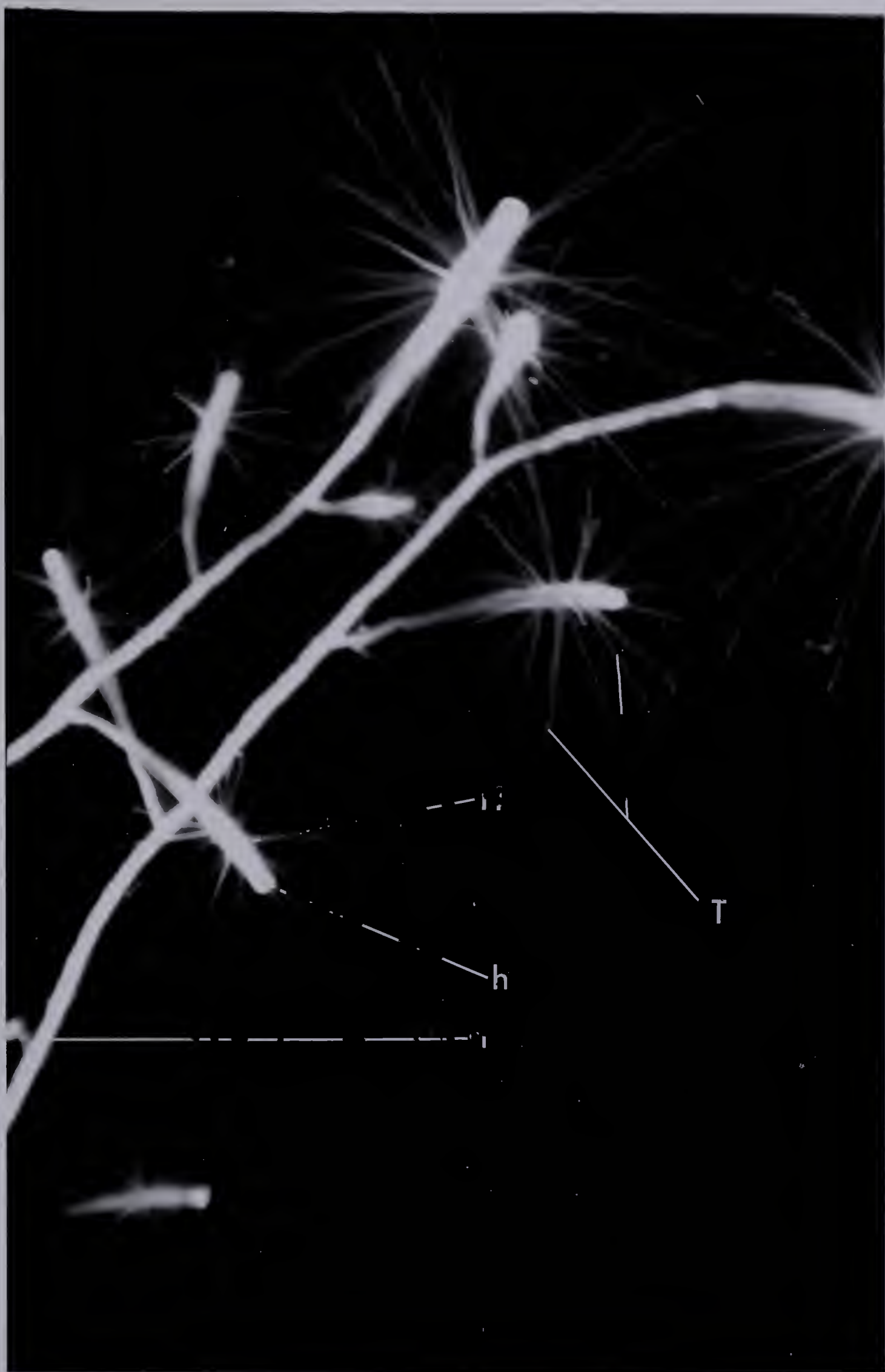
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STUDY OF THE NERVOUS SYSTEM IN CORDYLOPHORA LACUSTRIS

BY LIGHT AND ELECTRON MICROSCOPY

by

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A THESIS

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FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read,
and recommend to the Faculty of Graduate Studies for
acceptance, a thesis entitled "Study of the nervous system
in Cordylophora lacustris by light and electron microscopy,"
submitted by Raj Kumari Jha in partial fulfilment of the
requirements for the degree of Doctor of Philosophy.

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Date Nov. 16th, 1965

ABSTRACT

The identification of nerve cells in electron micrographs of osmium-fixed Cordylophora lacustris was achieved by comparison of the pictures so obtained with electron and light micrographs of the material in which the nerve elements had been selectively impregnated with silver.

The nerve cells and their processes were found only in the ectoderm of all the major regions of the hydranth and the tentacles but not in the stolon. Nerve cells are located intra- or subepithelially and the relative abundance of mono-, bi-, tri- and tetrapolar cells is 8%, 70%, 17% and 5% respectively. Total visible length of nerve processes is approximately 100 - 200 μ per nerve cell body, and they are mainly orientated longitudinally. They contain type-A and small type-B vesicles and occasionally a few ribosomes.

Most of the nerve cells carry a sensory hair, subepithelial or projecting externally, which shows a typical ciliary structure and usually arises from the base of a pit.

The nerve cells are considerably smaller in size than the epithelio-muscular cells. The nucleus usually contains a nucleolus and lacks the protein or the fibrillar bodies often found in ectodermal nuclei.

In addition to an elaborate Golgi complex and mitochondria, nerve cells usually contain three types of vesicles. Type-A, vesicles containing an electron-dense granule in the centre, and characteristic of nerve cells only. Type-B, vesicles lacking the granules found in type-A and showing variable sizes. They are roughly categorized

into "small" and "large" B-type vesicles. The small ones are often found characteristically concentrated beside the nerve cell membranes. Large ones are more irregular in shape and fewer in number. Type-C, vesicles containing homogeneous but moderately dense material, mostly associated with Golgi complex.

Multivesicular bodies of unknown nature, lipid bodies, lysosomes, profile of rough-surfaced endoplasmic reticulum and occasionally free ribosomes are also found in the nerve cells.

A clear division into "nerve cells" and "sensory cells," or ribosome-rich, ribosome-poor and neurosecretory cells, such as described for Hydra, is not possible in the case of Cordylophora.

Nerve cells are often found closely associated with epithelio-muscular cells, muscle processes and cnidocytes but the physiological significance of these contacts is not known.

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I. INTRODUCTION

Since the discovery of a nervous system in Eudendrium by Jickeli in 1883 (Schneider, 1890) there have been numerous studies on the nerves in hydroids, most of which have been on Hydra itself (e.g. Schneider, 1890; Hadzi, 1909; Semal-Van Gansen, 1952). Though the results obtained by various workers were somewhat different, yet the existence of the nerves and the nerve cells was considered to be well established until the electron microscope studies on Hydra by Slautterback and Fawcett (1959), Hess, Cohen and Robson (1957) and Hess (1961a). They were unable to identify the nerve elements in their preparations of Hydra with the electron microscope, and doubts were even expressed about the nerve elements described by the earlier workers (Hess, 1961b). However, the light microscopists' efforts to reaffirm the existence of nerves in Hydra (e.g. Spangenberg and Ham, 1960; Mackie, 1961; Burnett and Diehl, 1964) continued, and recently progress has also been made with electron microscope (Lentz, 1963; Lentz and Barrnett, 1965) towards the characterization of the "nerve elements."

The present study represents an attempt to study, in detail, the nerve elements in Cordylophora lacustris, which is a relatively simple hydroid. Hydra seems to be one of the least suitable hydroids for the neurohistological study, because its body wall is histologically complex due to the presence of a large number of interstitial cells. The hydranth wall of Cordylophora lacks interstitial cells and is very suitable material for the study of the nerve elements.

In this study an attempt was made to bridge the gap between studies of hydroid nerve elements with the optical and the electron microscopes so as to provide certain criteria for the identification of the nerve cells under the electron microscope, as well as to describe, in particular, the nerve elements in Cordylophora.

The study has been carried out in three major steps:

1. study of the nerve fibres and the nerve cells with the light microscope, stained selectively with silver impregnation technique (Jha, 1965);
2. location and identification of nerve elements in silver stained preparations examined with the electron microscope, based on the specificity of the silver stain, and on shapes and distribution of the nerve elements in the light microscope study;
3. identification and characterization of the nerve elements seen in osmium-fixed material with the electron microscope compared with the location, distribution, size, shape and certain other characteristics of the nerve elements identified in the electron microscope study of the silver-stained preparations.

II. MATERIAL AND METHODS

Colonies of Cordylophora lacustris (Allman) were grown in the C.V.D. mixture as described by Fulton (1960, 1962), and were fed every day on freshly hatched Artemia larvae. With regular feedings and changes of culture water the colonies continued to grow indefinitely. However, occasional cleaning and removal of excessive growth was necessary to avoid overcrowding and maintain healthy colonies.

A. SILVER STAINING TECHNIQUE FOR STUDY WITH THE LIGHT MICROSCOPE

Whole mount preparations were made for this study using a modification of the method described by Peters (1955a). Also some stained pieces were embedded in paraffin and sectioned. The whole mount preparations were most useful, and all the photographs are based upon them except Fig. 5G, which is a tangential section, 10 μ thick. With good resolution, considerable cytological detail can be seen in these whole mounts.

1. Preparation for staining

(i) Before fixation, the hydranths were fed individually on pieces of thick Enchytraeus albinus. When the body wall was well expanded, the hydranths were cut off and dropped immediately into picroformol (3 parts saturated picric acid + 1 part formaldehyde) and fixed overnight (roughly 14 - 18 hours).

(ii) Hydranths were washed in running water for about 6 - 8 hours and the worms were removed from the hydranths during this period.

(iii) The hydranths were washed in distilled water for half an hour with 2 - 3 changes.

(iv) The hydranths were brought to 70% alcohol and stored in it for any longer period only after 3 - 4 initial changes of alcohol. (They should stay in 70% alcohol for a minimum of 24 hours.) Pieces of the well-stretched hydranth wall were cut out with fine scissors at this stage.

2. Staining

(i) The pieces (2 - 3 at a time) were washed in glass-distilled water for 1-1/2 - 2 hours with 3 - 4 changes at half an hour interval each.

(ii) They were transferred to the impregnating bath warmed beforehand to 45° C and were impregnated for 17 - 18 hours at this temperature in the following solution: 5 drops of 1% AgNO₃ to 100 ml of a solution consisting of 80 ml glass-distilled water and 20 ml borate buffer (M/5 boric acid and M/20 sodium borate; pH 8.00)(Jha, 1965).

(iii) The hydranth pieces were rinsed in 2% anhydrous sodium sulphite in glass-distilled water for 5 - 10 minutes.

(iv) They were washed in glass-distilled water for 1-1/2 - 2 hours with 3 - 4 changes.

(v) Then the pieces were transferred to half of the freshly made developer at pH 6.3 at room temperature without AgNO_3 for 2 - 3 minutes and subsequently developed in the other half of the same solution with 5 drops of 1% AgNO_3 /10 ml of the developer solution until the tissue was dark brown or just black. The developer was made up as follows:

Peters' "glycine" developer stock solution - 10 ml

0.1 M sodium citrate - 10 ml

0.1 citric acid - add drop by drop until

the mixture's pH reaches 6.3.

(vi) The pieces were washed in running water for 15 - 20 minutes.

(vii) Then they were rinsed in distilled water briefly, dehydrated in a graded series of ethyl alcohol, cleared in xylene and mounted in neutral Canada balsam.

B. SILVER STAINING FOR STUDY WITH THE ELECTRON MICROSCOPE

Silver stained pieces of hydranth wall were embedded in Araldite essentially as suggested by Luft (1961), according to the following procedure:

(i) The pieces of well-stretched hydranth wall were stained and dehydrated according to the above-given technique.

(ii) After absolute alcohol, instead of clearing in xylene, the hydranth pieces were transferred to propylene oxide, 2 changes of 5 - 10 minutes each.

(iii) Then they were transferred to a 50:50 mixture of fresh propylene oxide and plastic mixture. Plastic mixture:

DDSA (Dodecenyl-succinic anhydride) - 23 ml

Araldite 502 - 27 ml

Accelerator (Benzyl-dimethylamine) - 0.5 ml

(iv) After one hour about the same volume of fresh plastic mixture was again added and the material was left overnight in an uncovered vial in a dust-free place.

(v) The gelatine capsules (No. 00) with pre-cast platforms of plastic were filled with fresh mixture of plastic with accelerator from which all the air bubbles had been removed by evacuation.

(vi) The hydranth pieces were transferred to these capsules, one each, and left for a day at room temperature, before polymerizing them, to ensure good infiltration.

(vii) For polymerization the capsules were transferred to 45° C oven for overnight and then to 60° C oven for 2 - 4 days.

Most of the blocks were re-orientated as desired, after polymerization. For re-orientation the bottom of the capsule with the hydranth piece in it was cut out by a small saw, shaped and

orientated and was then glued on to the top of the "blank" with Devcon clear epoxy adhesive mixture.

Longitudinal, cross and tangential sections were cut with a Porter-Blum microtome (MT-2) varying from 600 - 1000 Å in thickness. Freshly cut glass knives were used every time for sectioning. The sections were collected on 1 - 5 percent solution of acetone in distilled water, expanded by xylene vapours, and were picked up on uncoated copper grids (150 - 200 mesh).

For serial sections brass grids, with diameter = 3 mm, central hole = 1 mm in diameter, and thickness - 0.3 mm, were used. To support the sections the grids were double-coated with a celloidin and a carbon film. A drop of 1% celloidin in amylacetate, spread over chilled water to make a thin film, was used to make celloidin film on the grids which being fairly elastic is resistant to the manipulation of mounting and drying of the sections. The carbon film used at the top of the celloidin film makes the film stable in the beam.

Philips EM 100B electron microscope, with an objective aperture of 25μ, was used for this study. Electron-micrographs were taken on 35 mm Kodak film (P 426) at initial magnification of 1400 to 9000. These were further enlarged photographically 6 to 9 times.

A saturated solution of uranyl acetate in distilled water was used for counterstaining.

C. OSMIUM-FIXATION FOR ELECTRON AND LIGHT MICROSCOPE STUDY

Four different fixatives were tried. These were:

(1) 2% osmium tetroxide solution in distilled water buffered by veronal acetate buffer to pH 7.4, to which sucrose had been added; at 4° C for 1 - 1-1/2 hours (Palade, 1952; Caulfield, 1957);

(2) 1.3% osmium tetroxide buffered with S-collidine (2, 4, 6-trimethyl pyridine) buffer to pH 7.5, at 4° C for 1 - 1-1/2 hours (Bennett and Luft, 1959; Wood and Luft, 1965);

(3) 1.5% osmium tetroxide solution in C.V.D. culture water buffered with S-collidine buffer to pH 7.65, at 4° C for 1 - 1-1/2 hours;

(4) 3% glutaraldehyde in Sorenson's phosphate buffer at pH 7.3 and temperature 4° C for 2 - 3 hours, with post-fixation in 2% osmium tetroxide solution in same buffer and same temperature for 1 - 1-1/2 hours (Sabatini et al., 1962, 1963).

The material after fixation with glutaraldehyde was washed for 2 hours (4 changes each at 30 minute interval) in buffer with 10% sucrose, at 4° C. This washing before post-fixation with osmium is very important to avoid reduction of osmium with glutaraldehyde.

In all cases both stretched (as described earlier) as well as unstretched normal hydranths were fixed. Pieces of stolon with or without hydranths were also fixed.

Dehydration

Ethanol was used for dehydrating the material at 4° C after fixation according to the following schedule: 30%, 50%, 70% and 90% ethanol, each for 15 minutes, followed by 4 changes of 100% ethanol, for 10 minutes each.

The worms from the stretched hydranths were removed in 50 or 70% ethanol. The material in absolute ethanol was allowed to come to room temperature before transferring it to propylene oxide. Embedding, sectioning, etc., were carried out in the same way as described earlier for silver stained preparations for study with the electron microscope.

The sections on the grids in this case were also usually stained with saturated uranyl acetate solution in distilled water, which gave good results. Phosphotungstic acid staining was used on one occasion, but the contrast obtained was no better than with uranyl acetate and the preparations appeared to be contaminated. So the reagent was not used subsequently.

For study with the light microscope, sections roughly 3000 - 7000 Å thick were cut, mounted on a clean glass slide by drying them on a hot plate. They were stained with toluidine blue or methylene blue, washed with distilled water, dried and mounted in D.P.X.

III. OBSERVATIONS

In presenting these observations frequent references will be made to the findings of other workers in this field, and the new findings will be discussed in relation to those previous observations at appropriate points. It is hoped that a clear picture will thus be given of the essential background to this study and that the reasons for, and significance of the new work will become clearer than if the discussion was entirely relegated to a later section.

1. GENERAL HISTOLOGY OF CORDYLOPHORA

A colony of Cordylophora consists of stolon and the hydranths (Frontispiece). In each hydranth three regions can be distinguished, namely, tentacles, hypostome or the mouth region and the main hydranth or the gastric region. Cordylophora has two cellular layers, ectoderm and endoderm separated by an acellular layer, mesoglea (Fig. 22A). In the hydranth and tentacles, the ectoderm is mainly composed of epithelio-muscular cells and contains dispersed cnidocytes and nerve cells. The endoderm is composed of digestive cells and gland cells (Fig. 23). The muscle processes of the ectoderm and the endoderm are usually located adjacent to the mesoglea and are orientated longitudinally in the ectoderm and transversely (circularly) in the endoderm (Fig. 24). The height and the general appearance of the ectoderm and the endoderm of the hydranth wall varies slightly, depending on the state of expansion or contraction, however this does not apply in the case of the

tentacles. Sections of the stalk usually show young cnidoblasts (Figs. 49 and 43), which are rich in endoplasmic reticulum and contain young developing nematocysts. Interstitial cells are not found in the hydranth or the tentacles and appear to be differentiating in the region of the basal stalk. The hydranth once formed, lives for considerable periods. Kinne (Fulton, 1962) found that individual hydranths of Cordylophora live for at least 140 days. During this period the animal is using up nematocysts and presumably replenishing them by upward movement of the cells from proliferation region below the hydranth.

The structure of the various components of the ectoderm and the endoderm is discussed later, in the description of the nerve elements, and is compared with that of the nerve cells and their processes.

2. DESCRIPTION OF THE NERVE ELEMENTS

None of the classic papers on Cordylophora gives a clear idea of the character of the nerve elements. Indeed, very few descriptions even refer to the nerve cells. Some workers appear to have confused interstitial cells with the nerve cells (Tessonow, 1959). A short preliminary account by Mackie (1961) is the only source of information regarding the general appearance and distribution of the nerve elements in this form.

A. Results in the Study with the Light Microscope

The location, distribution and structure of the nerve elements has been studied in the silver-stained whole mount preparations of Cordylophora.

(i) Location

Nerve elements in Cordylophora have been seen regularly in the ectodermal layer. Throughout the study they were never seen in the endoderm contrary to what has been described in other hydroids by some of the earlier workers, e.g. Schneider (1890) in Hydra, Tubularia and Eudendrium; Semal-Van Gansen (1952) in Hydra and von Lendenfeld (Hadzi, 1909) in Eucopeella campanularia. According to Schneider (1890) in Hydra the ectoderm has only ganglion cells but no sensory cells and it is only the endoderm which has sensory as well as ganglion cells. Leghissa (1950) saw few sensory cells in the endoderm of Tubularia. Hadzi (1909), though, also found few sensory cells in the maceration preparation of the endoderm of Hydra, but he himself seems to have been very dubious about their presence in this layer, as he remarks that they might have come from the ectoderm, becoming accidentally mixed up with the endodermal cells.

In Cordylophora Morgenstern (1901) and Pauly (1902) demonstrated isolated cells, distinguished by small nuclei and long processes, located at the base of the ectoderm or in the mesoglea, which they called "Ganglienzellen."

(ii) Distribution

a. Hydranth:- I have found that the nerve cells are abundant throughout the entire ectoderm of the hydranth of Cordylophora. In the hydranth wall they are fairly uniformly distributed ranging from 4 - 7 nerve cell bodies/ $(100\mu)^2$. Local concentration of the nerve cells in the oral region was not seen contrary to what has been described for Hydra by Hadzi (1909) and Marshall (1923). McConnell (1932) also illustrates a nerve net with a high concentration of nerves in the hypostome region but Semal-Van Gansen (1952) believed that the pedal disc was the only region where they were concentrated. According to Spangenberg and Ham (1960) these variations in results may be due to the variations in the techniques used because with vital methylene blue staining technique nerves stain in some parts first and in other parts later. No such problem is encountered with silver staining technique because all the nerve cell bodies and the processes stain at the same time with almost the same intensity. Burnett and Diehl (1964) recently, using vital staining have again described concentration of ganglion cells in the hypostome and the basal disc regions of Hydra, and according to them they are either lacking or very few in number in the gastric region. In Cordylophora I have found no such concentrations comparable to those described in Hydra; this could be related to the fact that the tentacles are present all over the hydranth and are not localized in a ring around the oral region as in Hydra. The question of a concentration in the pedal disc does not arise in the case of Cordylophora because unlike Hydra it is a sessile colonial hydroid.

b. Tentacles:- I find the number of the nerve cells in the tentacles to be slightly lower than in the hydranth wall, which agrees with Semal-Van Gansen's observations in Hydra, though there is a fairly rich supply of nerves in the tentacular bases. Pauly (1902) did not see any nerve cells in the tentacles of Cordylophora either in the sections or in his methylene blue preparations, whereas Marshall (1923) describes tentacles as one of the regions with highest concentration of the nerve elements, in Hydra. Burnett and Diehl (1964) and most of the other workers, however, have described nerve cells in the tentacles of Hydra and some other hydroids. In Tubularia preparations stained with vital methylene blue technique (Jha, unpublished observations) several nerve cells were seen in the tentacles.

c. Stolon:- During this study, contrary to Mackie's finding (1961) no nerve cells have been seen in the stolon.

(iii) Structure

a. Nerve cell bodies:- The size and shape of the nerve cells is very characteristic and they are fairly easily distinguishable from other tissue elements in Cordylophora. They are the only cells with long, distinct and branched processes. Their nuclei are smaller than those of the epithelio-muscular cells and are oval or round. The nerve cell nuclei often seem to contain a nucleolus (Figs. 5C and D) which, however, is usually not very distinct in these preparations, as the whole nucleus is quite densely stained with silver. Schneider (1890) describes nerve cell and sensory cell

nuclei of Hydra as without nucleoli. According to Burnett and Diehl (1964) ganglion cells of Hydra are characterized by the lack of a nucleolus and the presence of densely staining droplets in the nucleus. In Tubularia nerve cells stained with methylene blue the nucleolus was invariably seen (Jha, unpublished observations).

The protein body which occurs within the nuclei of the epithelio-muscular cells (Mackie et al., 1964) has never been seen in the nerve cell nuclei.

Among the neurones I have found mono-, bi-, tri-, or tetrapolar forms (Figs. 2, 4 and 5). In the hydranth wall bipolar cells seem to predominate (Fig. 1). On the basis of a count of 100 nerve cells the relative abundance of the different forms in the hydranth wall was found to be:

monopolar	8%
bipolar	70%
tripolar	17%
tetrapolar	5%.

Leghissa (1950) and Semal-Van Gansen (1952) believed that the multipolar cells predominate in the ectoderm throughout the hydranths of Tubularia and Hydra respectively. Bipolar cells, according to Leghissa, are especially evident in the proximal tentacles and along the manubrium of Tubularia, running parallel to each other. My own observation in Tubularia is that the bipolar cells predominate in all regions, usually running parallel to the longitudinal axis of the region concerned.

In my preparations of Cordylophora bipolar nerve cells are found running in all directions, though the predominant orientation is more or less longitudinal to the axis of the hydranth (Fig. 1). Also these bipolar cells have been found usually to be lying parallel to the mesoglea as described by Hadzi (1909) in case of Hydra.

It seems very difficult to distinguish clear categories of nerve cells in Cordylophora corresponding to those set up for Hydra by various authors. Miyashima (1898) distinguished two types of nerve cells: (1) larger ones which are connected with each other by processes into a plexus-like structure and are called by him "central nerve cells" and (2) the smaller ones, which he calls, "peripheral nerve cells." Recently Burnett and Diehl (1964) have also distinguished two kinds of ganglion cells in Hydra, viz. small bipolar and large bi- or multipolar cells. But in the case of Cordylophora there is no such differentiation of nerve cells based on their size.

Hadzi (1909) has described three categories of nerve cells: (1) typical nerve cells, (2) neurosensory cells and (3) typical sensory cells. According to him "typical nerve cells" are located among the bases of the epithelio-muscular cells and lie parallel to the mesoglea. The "typical sensory cells" lie between the cell bodies of the epithelio-muscular cells more or less at right angles to the mesoglea. They have a long and narrow cell body with two basal processes and a fine sensory hair at the free end projecting

beyond the surface. The "neurosensory cells" lying at various heights in the epithelium, may or may not reach the outer surface. They may have more than two processes and no sensory hair is described at their free end.

In Cordylophora I have found all the types of the nerve cells comparable to those described by Hadzi in Hydra, but in addition there are many nerve cells which show variant characters and are often intermediate between his types; e.g. bipolar nerve cells with subepithelial sensory hair (Figs. 2A and H, and Fig. 4A), monopolar cells with a sensory hair at the tip (Figs. 2K, 5C and 5E), bi-, tri- or tetrapolar nerve cells with a long process carrying a sensory hair at its tip but with short compact cell bodies otherwise resembling "typical nerve cells" (Figs. 2B and 2I and 4B and 4F). Burnett and Diehl (1964) have also described distinct ganglion and sensory cells in the case of Hydra, the two being distinguishable by the absence and presence of sensory hair respectively. In my Cordylophora preparations nerve cells have occasionally been found which have no sensory hair (Figs. 2E, 4C, nerve cell with an arrow pointing towards it), and might be considered as "typical nerve cells" in Hadzi's sense.

One out of 8 - 10 nerve cells in Cordylophora has a sensory hair projecting externally, either carried on the tip of a long process (Figs. 2B, 2I, 4B and 4F) or on the tip of the nerve cell body itself (Figs. 2J, 2K, 2L, 4D, 5C and 5E). The subepithelial sensory hair is a very well defined structure (Figs. 4A, 5A, 5D and 5G),

and can be, occasionally, $2.5 - 3\mu$ long, projecting into the surrounding area. In Cordylophora only one sensory hair, whether subepithelial or projecting externally, has been found per nerve cell body in contrast with 1 - 5 (McConnell, 1932) or 1 - 3 (Burnett and Diehl, 1964) sensory hairs described in Hydra's "sensory cells." Another hydrozoan Physalia shows 2 or more sensory hairs per nerve cell and in this case also, like Cordylophora, the majority of the nerve cells possess these hairs (Mackie, 1960a). It seems that there is considerable variation in different hydrozoans in this regard.

Mackie (1961) suggested that the cells with subepithelial sensory hair might be modified sensory elements, which have become or are becoming transformed into "nerve cells" in the strict sense, or that they are functional sensory cells serving to record deep touch or to give the position sense.

Hertwig and Hertwig (1878, 1880) long ago pointed out that the sensory cells of coelenterates do not always reach the surface, their cell bodies can be sunk more or less deeply into the epithelium, and their peripheral sensory processes need not necessarily project beyond the epithelial surface. Their basal processes, according to them, can resemble those of the "typical nerve cells" and the two may thus be indistinguishable. From the existence of such intermediate stages the Hertwigs speculated that the nerve cells had developed phylogenetically by the sinking in of the sensory elements.

However, according to McConnell (1932) who studied the development of the nervous system in the buds of Hydra with methylene blue, both nerve cells and the sensory cells arise from the interstitial cells independently. McConnell claims that the sensory cells after being differentiated from the interstitial cells reach the ectodermal surface, develop two processes at the proximal end; the distal end on reaching the external surface develops the characteristic sensory hair. This would indicate that both nerve and sensory cells are produced alike from the interstitial cells, and that the sensory cells are those nerve cells which have moved up to the surface and have developed a sensory hair. This hypothesis regarding the ontogeny of the sensory cells is the complete opposite of the phylogenetic sequence proposed by Hertwigs (1878). If the sensory cells arise from interstitial cells as McConnell says, then cells with subepithelial sense hairs, in Cordylophora, could be the immature sensory elements which have developed a sensory hair before reaching the external surface, rather than being the sunken ones. In a mature hydranth a cell growing up might well resemble a cell sinking down. Judging from the fact that the majority of the nerve cells have a subepithelial sensory hair, however, one can say that such cells are not the intermediate stages, instead they are the fully developed functional nerve cells which, in addition to the functions of "typical nerve cells" may be carrying out certain sensory functions of the type suggested by Mackie (1961).

b. Nerve cell processes:- In the present study, it is found that all types of the nerve cells, whether they have a sensory hair or not, have similar processes which may or may not branch. No classification of processes along the line of Semal-Van Gansen's (1952) scheme for Hydra seems possible in case of Cordylophora. In Hydra Burnett and Diehl (1964) also describe small nerve cells with "axons" up to 15μ long and large nerve cells with "axons" from $30 - 200\mu$ long. In their preparations of Hydra stained vitally with methylene blue they also distinguish "axons" in various regions of Hydra depending on the presence or absence of the swellings and the densely staining droplets in them, none of which have ever been seen in the silver-stained preparations of Cordylophora. However, in my opinion the micrographs published by Burnett and Diehl (1964) do not show detailed structure of the nerve cells with clarity, perhaps due to the limitations of the methylene blue technique.

The total visible length of the nerve cell processes originating from one nerve cell averages $100 - 200\mu$ (cf 1000μ in Forskalia (Mackie, 1965)), but since the finest terminal branches are often very hard to follow, this estimate may be on the low side. The nerve processes decrease in thickness distally from the nerve cell bodies. Though the majority of the nerve processes in the hydranth wall and in the tentacles are orientated more or less in the longitudinal direction (Fig. 1 and the histogram in Fig. 3), a few running across can also be seen occasionally in the hydranth wall.

B. Results of the Study with the Electron Microscope

For the purpose of characterization and identification of the nerve elements in the osmium-fixed preparations of Cordylophora, this study was carried out in two steps.

(i) Comparison of light and electron microscope study of silver stained preparations

a. Shapes of the nerve elements and their distribution:-

The picro-formol fixative that alone gave good results in the silver-staining technique, did not prove to be good for the preservation of the cytoplasmic organelles like mitochondria, Golgi complex, endoplasmic reticulum, etc., for the purpose of electron microscope study. However, the relative position and the limiting boundaries of the various tissue components were preserved fairly satisfactorily. Shapes of the cell bodies, nuclei, protein bodies, nucleoli, etc., were not distorted and all the correlative details required for the present study were adequately shown (Fig. 6, 9 and 15). Fixatives which show the cytoplasmic details well under electron microscope could not be used in conjunction with specific silver staining of the nerve elements with the possible exception of formalin or glutaraldehyde. Attempts to silver stain the material fixed with formalin or glutaraldehyde (without post-fixation in osmium) were unsuccessful. Osmium tetroxide is quite incompatible with silver staining by this method, because combination of osmium with the reducing groups in the tissue would inhibit the uptake of silver.

Acrolein was tried, also without success. Acrolein in combination with picric acid and formalin mixture was not attempted and might prove useful.

The nerve cells in the silver-stained electron microscope preparations were distinguishable from the other structures with reasonable certainty and they have been found only in the ectodermal layer. Similar structures have never been found in the endoderm during this study, as also mentioned earlier in the description of the light microscope study. General shapes, dimensions and certain specific characters of these nerve cells correspond to those of the ones seen in the light microscope preparations. Since it is only rarely possible to get a perfect section of a nerve cell passing through the processes as well as through the cell body in the region of the sensory hair, serial sections frequently proved necessary.

Since the cell membranes are not well preserved in these preparations, it is often difficult to trace the exact location of the nerve cells, in relation to the epithelio-muscular cells, however, it appears that they are either sub- or intra-epithelial. The nerve cells are much smaller in size than the ectodermal cells. Their cytoplasm is usually rather compact whereas the ectodermal cells are highly vacuolated. The nuclei of the nerve cells are quite distinct from those of the ectodermal cells regarding their size, absence of the protein body and the relative density of the silver deposition (Figs. 15 and 16). The nerve cell nuclei are usually oval or round and invariably contain a nucleolus (Figs. 6, 10 and 16),

unless occasionally when the section does not pass through it. The silver deposition in the nucleolus is much heavier than in the nucleus itself.

Corresponding to the observations in the light microscope study both the types of sensory hairs, i.e. subepithelial (Figs. 7, 9 and 25) as well as ones projecting externally (Fig. 18) are found in these sections of the nerve cells. In these preparations they have almost always been found either surrounded by a pit or arising from the base of a similar pit, which is approximately 600 - 1000 m μ wide. During the light microscope study a similar pit at the base of the sensory hair was quite distinctly seen in one bipolar nerve cell (Fig. 4A), however, in most cases it was not clearly visible. Burnett and Diehl (1964) in their light microscope study of nerve cells of Hydra have described a small "vacuole" just below the sensory hair of, what they call, "sensory cells." The sensory hair in Cordylophora nerve cells is roughly 250 - 400 m μ thick, and is very densely stained with silver. The process carrying the sensory hair at its tip, as seen in the light micrographs (Figs. 4B and 4F) is also seen in some electron microscope preparations (Fig. 18) showing some silver deposition in it, around the base of the cilium.

The cytoplasm of the nerve cell bodies which appears relatively lighter in the light microscope preparations, as compared with the nerve cell nuclei, nerve processes and the sensory hairs, shows much less silver deposition.

The nerve cell processes are often seen in the form of isolated sections lying in the ectoderm and occasionally along with the nerve cells (Figs. 10, 11 and 12). The thickness of the nerve processes is very variable depending on the distance from the nerve cell body where they originate, since they are thickest at the base and taper distally, as also described in the light microscope study. They vary roughly from 600 - 1000 m μ in thickness near the nerve cell bodies; and sometimes sections of nerves varying from 80 - 100 m μ or less have also been seen. Occasionally the pieces of nerve processes look much thinner even at the base near the nerve cell body itself, in which case it seems, apparently the section is passing only through a part of it. The density of silver deposited on the nerve processes is even more than on the nucleolus (Fig. 47). Judging from the study of the sections with light microscope (Fig. 5C) and the electron microscope (Figs. 13 and 14) it appears that the heaviest silver deposition is on the surface of the nerve processes. With regard to distribution, the nerve cells have been found in ultra-thin sections of silver stained material, taken from all major regions of the hydranth, as well as in the tentacles. The local variations in density are discussed elsewhere (p. 13).

b. Specificity of the silver deposition:- The silver staining technique used for the light and electron microscope study of the nerve elements of Cordylophora has proved to be very useful and selective for this purpose; however, slight variations in pH, temperature, concentration of silver in impregnating and developing mixtures, time of impregnation and development, and contamination

of the distilled water or any of the chemicals used, have occasionally been responsible for the unsuccessful results. When selectively stained, the preparations are very clean, with very little, if any, unspecific deposition of silver outside the tissues, as has been seen in both light as well as electron microscope preparations.

Mackie (1960b) and Batham, Pantin and Robson (1961) have described structures other than the nerve elements, e.g. flagella, discharged nematocyst filaments, muscles, mesogleal tissue, certain membranes, spindle fibres, nuclei and nucleoli of cells other than the nerve cells, which can often be stained with silver, when the technique for staining nerve elements selectively, is not followed accurately, and in some cases even in the specifically stained preparations. In the selectively stained preparations of Cordylophora in the light microscope study, the only structures in addition to the nerve elements which are relatively densely stained with silver are ectodermal and endodermal nuclei, cnidocytes and discharged nematocyst threads. The ectodermal cell walls are hardly visible in the whole mounts. In both the light- and electron-micrographs of the silver stained preparations all these densely stained structures can be distinguished from one another with reasonable certainty.

Ectodermal and endodermal nuclei:- The ectodermal nuclei usually vary from 5.0 to 7.0 μ in diameter as compared with about 2.5 - 4.0 μ diameter of the nerve cell nuclei (Figs. 4 and 5). The endodermal nuclei may be as large as 9 μ , and are often larger than those of the ectoderm. These nuclei are characterized by the

presence of a protein body stained with silver only at its periphery (Fig. 16), which has never been seen in the nerve cell nuclei. The density of silver is approximately 100 - 110 grains/ μ^2 in the ectodermal nucleus as compared with 125 - 130 grains/ μ^2 in nerve cell nucleus. The size of an average grain of silver also varies from 40 to 50 m μ in the nerve cell nuclei and from 25 to 30 m μ in the ectodermal nuclei. In Guillery and Ralston's (1964) electron micrographs of the axon terminals in the cat spinal cord stained with Nauta silver technique the average grain size varies from 28 to 45 m μ and Peters (1955b) has described particle size varying from 3 - 70 m μ , however the size of an average particle, even in his preparations seems to be fairly close to that of those in Cordylophora nerve cells.

Both ectodermal and nerve cell nuclei have nucleoli, the diameter of which is typically about 1500 - 2000 m μ in diameter in ectodermal nuclei and 600 - 800 m μ in nerve cell nuclei. The density of silver in the nucleoli of both seems to be almost the same.

Cnidocytes and discharged nematocysts:- In the light-microscope preparations the cnidocytes are distinguishable from the nerve cells by the presence of the characteristic capsule (cnidocyst) (Figs. 5C and 5D) and the absence of the basal processes found in the nerve cells. There is not much difference in the size of the nuclei of the cnidocytes and the nerve cells. Sometimes in the electron microscope preparations of the silver-stained material, however, the nuclei of the two look different (Fig. 15). The nuclei of the cnidocytes are full of electron dense material quite distinct

from the silver particles, and contain much less silver than do the nerve cell nuclei. The nematocysts are well preserved in these preparations (Figs. 15 and 17) and the outer wall of the capsule sometimes shows a deposition of silver on it, as seen in Fig. 15. Though the sensory hair of the nerve cells and the cnidocil of the cnidocytes are both very densely stained with silver, yet the latter can be easily identified by the presence of the characteristic spirally arranged supporting structures around it, and thus can never be confused with the former (Figs. 18 and 19). Similar supporting structures around the cnidocil of Hydra have been reported earlier by Chapman and Tilney (1959) and Slautterback (1961). In the cnidocil the silver deposition is only at the surface and there is almost none in the core (Fig. 45). Sensory hairs, however, seem to be stained uniformly (Figs. 8 and 9).

The discharged nematocyst threads have been seen in the light microscope preparations, darkly stained with silver, but they are always at a different focal level. They are usually on the outer surface, and have never been seen in the level of the nerve processes as it is obvious from the micrographs. The nematocyst threads have rather a wavy course and also their origin from the capsule is very characteristic. The continuation of a nerve process from a nerve cell is very distinct and unmistakable (Figs. 4, 5, 10 and 11).

The first of these is the fact that the government has not yet decided whether it will accept the offer of the United States to provide a loan of \$100 million to the United Kingdom. This is a very important decision, as it will determine whether the United Kingdom will be able to meet its obligations to the United States. The second of these is the fact that the government has not yet decided whether it will accept the offer of the United States to provide a loan of \$100 million to the United Kingdom. This is a very important decision, as it will determine whether the United Kingdom will be able to meet its obligations to the United States. The third of these is the fact that the government has not yet decided whether it will accept the offer of the United States to provide a loan of \$100 million to the United Kingdom. This is a very important decision, as it will determine whether the United Kingdom will be able to meet its obligations to the United States.

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The muscle processes and the mesoglea:- I have not seen either of these in the silver-stained whole mount preparations under the light microscope. In the electron microscope preparations both are clearly visible and distinguishable from each other. Both have some deposition of silver (Fig. 20). The pattern of silver deposition in muscle is quite regular as it is seen in the oblique tangential sections at higher magnifications (Fig. 21). The question of confusing any of these two with the nerve elements does not arise because of the difference in the density and the pattern of silver deposited in them and their general structure.

(ii) Comparison of silver-stained and osmium-fixed electron microscope preparations

(a) Shape and structure of the nerve cell bodies:- For comparison with silver stained preparations the sections of the osmium-fixed hydranths in the stretched condition were studied first to identify the nerve cells and later nerve cells with similar characters and structure were also identified in the unstretched normal hydranth preparations.

Of all the fixatives tried, 1.5% OsO_4 in C.V.D. culture water buffered with S-collidine buffer to pH 7.65 proved to be the most useful one, and most of the electron micrographs are based on the preparations using this fixative. Glutaraldehyde with post-fixation in osmium also preserved most of the cytoplasmic organelles well (Fig. 33) but the fixative with sucrose caused some general shrinkage of the cytoplasm (Fig. 28).

the external surface can be disregarded because such cilia in whole mount preparations have never been found to be larger than 2.0 - 3.0 μ . The distance between the external surface and the point of origin of such a hair has been found to be occasionally 4 - 4.5 μ (Fig. 27) in electron microscope preparations.

Sensory hairs:- The sensory hairs are always seen arising from the base of a deep invagination in the cell surface which has been described as a pit in the description of the silver-stained preparations. In cross section the sensory hair appears to be located in the centre of an empty space.

The sensory hairs have not been studied at the highest resolution but they probably have the usual ciliary subunits. It is certainly clear that the two central tubules are present (Fig. 28) contrary to the situation in some non-motile sensory hairs of ciliary origin (Miller, 1958; Eakin and Westfall, 1960; Gray, 1960; Tokuyasu and Yamada, 1959). However, in the case of coelenterates, the presence of the central tubules in the sensory cilia has often been reported (Eakin and Westfall, 1962; Horridge, 1965; Lentz and Barnett, 1965). Recently Reese (1965) has found a similar situation in the olfactory cilia of frog.

The nine peripheral tubules merge at the base into the basal body (Figs. 31 and 32). A deposition of electron dense grains, which may be reduced osmium, has often been found in the cytoplasm around the base of the cilium and the ciliary shaft. In some examples such a deposition is much heavier at the top of

the basal body (Figs. 30, 31 and 39) but in others it was not so (Figs. 29 and 32). The lower part of the basal body, the centriole (Figs. 29, 32 and 33), is associated with a rootlet (Figs. 29 and 32). In one preparation, where the section passes longitudinally through the rootlet, it is approximately 3μ long, reaching almost half the length of the nerve cell itself (Fig. 32). The rootlet has a banded structure with alternating light and dark bands. Also associated with this root apparatus, a second centriole has occasionally been seen in cross section (Fig. 32). Similar structure at the ciliary root has been described by many other investigators, e.g. Batham (1960), for the cilium of an epithelio-muscular cell of the retractor muscle of Metridium canum, Gray (1960) for the sensory cilium in the locust ear where the banded root splits repeatedly into thirty or more rootlets; and by Westfall (1965) for the flagellum of the nematocyte of Metridium senile.

Horridge and Mackay (1962) have shown a similar banded rootlet in their micrograph of the outer region of the ciliated sensory cell. In the case of Hydra such a banded rootlet has not been described at the base of the sensory cilium; instead, according to Lentz and Barrnett (1965) from the base of the basal body, small dense filaments about the diameter of the microbubules, splay out into the apical cytoplasm.

In Cordylophora I have found these sensory cilia to be one of the most characteristic features of the nerve cells, as such cilia have never been found in any of the other tissues in the

The nerve cells were identified in the ectoderm by their resemblance to the nerve cells identified in the silver preparations (e.g. Figs. 25 and 26). These nerve cells are situated intra-epithelially amongst the cnidocytes, or subepithelially but above the muscular processes. As described earlier, in Cordylophora, the nerve cells invariably could be recognized, apart from their size and shape, by the presence of a sensory hair. This may be subepithelial (Figs. 22, 26 and 27) or projecting externally (Figs. 22, 29, 30, 31 and 32) except occasionally when the section does not pass through the nerve cell in the region of the sensory hair, or it may be lacking.

According to Lentz (1963) and Lentz and Barrnett (1965) the nerve cells in Hydra are of two types: (1) "ganglion cells" and (2) "sensory cells," the latter according to them are characterized by an apical specialization which is described as a modified cilium. Lentz and Barrnett (1965) also described a third type of nerve cells, i.e. "neurosensory cells"; these are deeply situated ganglion cells from the primary process of which a secondary process arises that contains a single cilium, which protrudes between the cells to reach the surface.

In my opinion, in case of Cordylophora such a distinction between various types of nerve cells does not exist, as has been shown also by light microscope study. In most cases deeply situated nerve cells, corresponding to the "neurosensory cells" of Hydra, show subepithelial sensory hairs and the possibility that they reach

ectoderm. Epithelio-muscular cells of the ectoderm do not appear to be ciliated. They, however, occasionally have small cytoplasmic projections or microvilli (Figs. 27 and 32, unlabelled) on their outer surface which do not show filaments or tubules like those of the sensory cilia. The cnidocils projecting from cnidocytes look very different from the sensory cilia due to the presence of the supporting structures (Figs. 40, 42 and 46) as mentioned earlier in the description of the silver preparations. Also, the cnidocils have a homogenous electron-dense core (Figs. 40 and 44) in contrast to a pair of tubules found in the centre of the sensory cilium.

In the endoderm, though gland cells and the digestive cells have cilia or flagella with similar $9 + 2$ pattern the general structure of these cells is very much different from that of the nerve cells (Fig. 38). Gland cells or the secretory cells are often full of large vesicles containing the secretion products. Towards the base, such cells show a rich endoplasmic reticulum, and several Golgi complexes. Small vesicles containing a dense material are observed at the ends of the parallel lamellae of the Golgi complexes, which may be secretory bodies. They appear to grow bigger as they move towards the periphery, where they are presumably released into the lumen (Fig. 23). The digestive cells contain varying numbers of unidentified inclusions of different kinds. They seem to be poorer in endoplasmic reticulum than the gland cells. In the endoderm there are several flagella per cell (Fig. 38) in contrast to one sensory cilium per cell in the case of the nerve cells.

Nuclei:- The nuclei of the nerve cells are usually oval or round, but they occasionally show irregular shapes. They are enclosed within a two-layered nuclear envelope. The membranes of the envelope contain irregular long spaces, about 50 - 200 m μ wide, which meet at intervals, where there is a nuclear pore (Fig. 35). The nucleus usually has a dense nucleolus and in addition several small dense patches of chromatin are present all over the nucleus and along the inner nuclear membrane. According to Lentz and Barrnett (1965) the nucleoli in Hydra nerve cells are not prominent. The nuclei of the nerve cells of Cordylophora differ from those of epithelio-muscular cells by the absence of protein bodies and of fibrillar bodies which are often found in the ectodermal nuclei (Fig. 37). The exact nature of these fibrillar structures is not known and they apparently escaped detection in the only previous electron microscope study on Cordylophora (Mackie et al., 1964).

Mitochondria:- Mitochondria have often been found in the nerve cells of Cordylophora, interspersed among other organelles in the cytoplasm. The number of mitochondria varies from one nerve cell to another, and occasionally several of them have been seen aggregated in any one part of the cell (Figs. 30 and 36). Mitochondria are usually round or slightly elongated, roughly 1 - 2 μ long and 300 - 700 m μ broad.

Golgi complex:- An elaborate Golgi complex has almost always been seen in the nerve cells. It is characteristically composed of a varying number of parallel membranes roughly 20 - 30 m μ

apart which appear to be forming vesicles at their ends by a pinching-off process. Numerous similar vesicles with diameters varying roughly from 50 - 100 m μ are usually found associated with the Golgi complex, which appear to have been formed and released from the ends of the parallel membranes. Sometimes, when the section is not cut parallel to the longitudinal axis of the membrane, a large number of small vesicles crowded together have been seen (Fig. 31).

Vesicles:- Vesicles, other than those closely associated with the Golgi complex, have also been found throughout the cytoplasm of the nerve cells of Cordylophora. However, they are not as crowded. These vesicles vary in form and can be divided into three categories:

Type A - These vesicles are characterized by the presence of an electron-dense granule in their central area and are usually round in shape (Figs. 29, 39 and 41). Similar vesicles have been described earlier in the sensory cells of the jellyfish, Cyanea (Horridge and Mackay, 1962) and in the "neurosecretory cells" of Hydra by Lentz and Barrnett (1965), who call them membrane-bounded dense granules. In Cordylophora these vesicles have been found in a number of nerve cells, which do not otherwise appear different or distinguishable from nerve cells which lack these vesicles. It is, therefore, doubtful if they should be considered to form a separate class, as suggested by Lentz and Barrnett (1965) in the case of Hydra. It seems very likely that the nerve cells with or without such vesicles represent a single type of cell in different physiological states. These vesicles in Cordylophora nerve cells vary roughly from 100 - 250 m μ

in diameter in contrast to the more uniform size (70 - 100 mμ) shown by similar vesicles in the sensory cells of Cyanea. These vesicles have not been found in any non-nervous cells. Occasionally vesicles of this type can be seen in the Golgi area (Fig. 41). Horridge and Mackay (1962) suggest that the granule in this type of vesicles is formed by invagination of the wall of the simple type-B vesicles, but no evidence for this suggestion was obtained in the present study. Similar vesicles have also been found in the nerve cells of other invertebrates (Rosenbluth, 1963; Coggeshall and Fawcett, 1964; Oosaki and Ishii, 1965). However, in these cases they are found in large numbers and are usually considered to be neurosecretory granules. In case of Cordylophora their number is rather small and the nature is not known.

Type B - These vesicles lack the electron-dense granule found in type A, and are of varied sizes. They can be roughly subdivided into two kinds although some intermediate forms have been observed. (1) Smaller ones which are often found concentrated adjacent to the nerve cell membrane, where the latter is in close contact with the other tissues, e.g. ectodermal epithelio-muscular cells (Figs. 30 and 34) or muscle processes (Fig. 36) or where no such cell is in the vicinity (Fig. 27). These vesicles vary in size roughly from 100 to 200 mμ in diameter and are more or less round in shape. They have also been found associated with the Golgi complex and scattered in the cytoplasm. (2) These are larger, more irregular in shape and fewer in number (Figs. 30 and 39).

Vesicles similar to these have also been found in the ectodermal cells and in the cnidocytes. Therefore, B-type vesicles are not diagnostic of nerve cells. However, it is only in the nerve cells that they have been seen regularly concentrated beside the cell membranes.

Type C - Homogeneous but moderately dense, small vesicles, mostly associated with the Golgi complex (Fig. 41).

Occasionally all the three types of vesicles have been found in a single nerve cell (Fig. 41).

Other cytoplasmic components:- Membrane-bounded multivesicular bodies of unknown nature have been found in the nerve cells of Cordylophora (Figs. 22, 31, 33 and 39), which may occasionally be present in the Golgi area (Fig. 31).

Lipid bodies have been found in some nerve cells (Figs. 35 and 41). A nerve cell in Fig. 39 contains lysosomes. Lysosome-like bodies seem to be present in other nerve cells also, but these have not been identified with certainty.

The endoplasmic reticulum of either smooth- or rough-surfaced kind, which most metazoan cells show, is not a very prominent feature of the nerve cells of Corylophora. I have seen profiles resembling rough-surfaced endoplasmic reticulum in some examples (Figs. 30, 33 and 35), but in other cells no such figures are seen and the cytoplasm appears to lack endoplasmic reticulum of either rough or smooth type (Figs. 26 and 39). The B-type vesicles

might represent portions of smooth endoplasmic reticulum, although this is unlikely since these vesicles are usually isolated from one another, and do not form a continuous system so far as can be seen. It is possible that differences reflect the fact that the nerve cells in question were sectioned through different regions. Local variations in type and form of endoplasmic reticulum are known in cells in other animals, e.g. mammalian liver cells. Free ribosomes have been found in the cytoplasm of some nerve cells in Cordylophora, but it seems very difficult to distinguish between ribosome-rich and ribosome-poor nerve cells as claimed in the case of Hydra (Lentz and Barrnett, 1965). Moreover sometimes the nerve cells containing free ribosomes do not show any A-type vesicles (Fig. 35) or vice versa, contrary to Lentz and Barrnett's observations in Hydra.

The microtubules found in the nerve cells of Hydra (Lentz and Barrnett, 1965) have not been seen in the nerve cells of Cordylophora in the routine or glutaraldehyde-fixed preparations. In preparations fixed with OsO_4 + sucrose, however, some structures vaguely resembling microtubules have been seen (Fig. 28), but since the general fixation of the cytoplasm is not very good in this case, it is very difficult to say anything definite about them.

(b) Distribution:- The nerve cells in Cordylophora have been found in all the major regions of the hydranth wall, as well as in the tentacles. In the hypostome region nerve cells with a sensory hair projecting externally have been found relatively more often than elsewhere in the hydranth.

Both cross as well as longitudinal sections of the stolon have been studied but without any success in finding a nerve cell. As mentioned earlier, nerve elements were not found in the endoderm.

(c) Description of the nerve processes:- Portions of the nerve processes lying in the spaces between the epithelial cells as seen in silver-stained material have been also found in osmium-fixed preparations (Figs. 26 and 29), or occasionally in direct contact with the mesoglea (Fig. 29). The nerve processes often contain type A and small type B vesicles (Figs. 26 and 48). Occasionally, however, a few ribosomes can also be seen.

Microtubules such as those found in the nerve processes of Hydra (Lentz and Barrnett, 1965) and Cyanea (Horridge and Mackay, 1962) have not been seen in the nerve processes of Cordylophora.

Lentz and Barrnett (1965) distinguish the neurites, belonging to the different types of nerve cells in Hydra, by the presence or absence of microtubules and dense granules. However, in Cordylophora, there appears to be no justification for establishing different categories either for the nerve cells or their processes.

The nerve processes are usually easily distinguishable from the muscle processes and mesoglea. The muscle processes do not contain A-type vesicles and their regular distribution and fibrillar structure is rather distinct (Figs. 22 and 24). The mesoglea is also a fibrillar structure (Fig. 49), which lacks its own limiting membranes and other structures such as mitochondria, vesicles or ribosomes. In cross section mesoglea appears granular (Fig. 24).

C. ASSOCIATION BETWEEN THE NERVE ELEMENTS AND THEIR
CONNECTIONS WITH OTHER ELEMENTS IN THE ECTODERM

In the light microscope study the nerve cell bodies have been found occasionally lying in pairs close to one another (Figs. 4C and 4E). During the electron microscope study of silver stained preparations also, such a situation was once encountered (Fig. 15A). Whether there is an actual fusion between the two cells, in these cases, is not very clear, since the silver stained electron microscope preparations do not show the distinct cell walls because of the fixation defect. Binucleate nerve cells described by Mackie (1961) have never been found throughout the study. In osmium preparations the nerve cells are often found lying beside or surrounded by epithelio-muscular cells with the cell membranes of which they are in immediate contact. In a section of a tentacle two nerve cells have been seen lying next to each other separated by a small bit of cytoplasm (Fig. 29). This cytoplasm most probably belongs to an epithelio-muscular cell. Nothing comparable to the synapses between axons (between axon and perikaryon) of Cyanea (Horridge and Mackay, 1962) or other invertebrates and vertebrates (De Robertis and Bennett, 1955; and Gray and Young, 1964) has been seen in Cordylophora. With the light microscope two or more nerurites were frequently seen running parallel to one another or twisted together over short or long distances (Figs. 1, 4C, 4G and 4H) as also described by Mackie (1961) in the hypostome region of Hydra. A similar tendency of nerves to run parallel is also reported by Batham (1965) in Mimetridium cryptum.

Relationships of the nerve cells with the other types of cells and structures are often not very definite or clear. With the light microscope the cnidocytes have been seen to lie in close contact with the nerve elements (Figs. 1, 5C). Nerve cells and cnidocytes have also been found in close contact with each other in silver stained electron microscope preparations (Fig. 9). In Fig. 9 there is comparatively a heavier deposition of silver at the point of contact between the two (pointed by an arrow). It is very difficult to comment on the exact nature of the specific deposition of silver at this point; however, it is suggestive of some kind of interaction between the two.

In the tentacles nerve cells have sometimes been found amongst the groups of cnidocytes (Fig. 15). In Fig. 29 a cnidocyte is seen lying adjacent to a nerve cell, and the cell membranes of the two are in intimate contact over a considerable distance; however, none of the structures associated with various vertebrate synapses such as vesicles, thickening of membranes, synaptic discs or infolding of the membranes has been seen. Spangenberg and Ham (1960) in their light microscope study of Hydra have reported specific nerve terminations on the cnidocytes, and later Lentz (1963) and Lentz and Barnett (1965) in their electron microscope study have also shown the processes of "ganglion" and "neurosecretory" cells in close association with the bases of the cnidocytes, but again there is nothing comparable to synaptic or neuro-muscular configurations of higher animals.

In the electron microscope study of Cordylophora, nerve cells and processes have often been seen in close contact with the ectodermal cells, their muscular processes and occasionally with the mesoglea. Sometimes almost the entire nerve cell-body has been found lying along side the muscular processes with, typically, a 25 - 50 mμ gap between them. Occasionally in such cases large numbers of small B-type vesicles in both muscle as well as the nerve cell have been seen at the point of contact (Fig. 36), but this cannot be regarded as evidence of a propagating junction. These vesicles are much larger than the true synaptic vesicles, and also none of the other features of the elaborate neuro-muscular junctions of vertebrates (Hess, 1965; and Karlsson, 1962) and other invertebrates (Horridge, 1965; and Reger, 1965) are seen.

IV. GENERAL DISCUSSION

One of the difficulties in recognizing the nerve elements in hydroids is their lack of a myelin sheath, and the absence of specialized structures at neuro-muscular and synaptic junctions. This has led to the doubts about the existence of these elements referred to previously. However, the nerves do show high specificity in silver stains like nerves in other animal groups and this has been used as a key in seeking to establish the identifying features of the nerves under the electron microscope.

The electron microscope study of the preparations stained specifically with silver, has served the desired purpose of identifying the nerve cells and their processes in conventional osmium-fixed electron microscope preparations and thus in correlating the light and electron microscopic observations.

This selective silver staining technique for the study of nerve elements with the light microscope has offered many advantages over the other techniques like maceration technique and vital methylene blue staining used by earlier workers. Proper fixation in the beginning of the technique helps to avoid any post-mortem changes in the tissue. The preparations are permanent and can be kept for a minimum of one year without any danger of deterioration of the tissue or the stain. It gives consistent results if employed carefully and accurately; it stains the whole tissue uniformly and simultaneously.

The silver staining method used for staining the nerve elements in Cordylophora did not give successful results with Hydra. Over a period of six months various modifications in fixation, impregnation and development were tried, which occasionally did stain nerve cells, but because of some background staining in these preparations, pictures showing distinct nerve cells could not be taken, and the information obtained was not worth including in the thesis.

Study of silver stained material under the electron microscope in addition to aiding recognition of the nerve elements serves the additional purpose of showing something of the location of the chief sites of silver reduction in the tissue.

The specificity of the silver stain seems to depend on the formation of silver "nuclei" during impregnation (Samuel, 1953) and this process, in turn, as in all other metallic impregnations, depends on the reducing capacity of the tissue. According to Wolman (1955) various tissues differ from each other and allow differential staining because of the factors given below.

1. The reducing groups may be either free, or may be formed or made available by different preliminary treatments.
2. The redox potential of the different tissues may be different.
3. The rate of reaction of the chemical reducing groups with different reagents may be different.

4. The amount of reducing activity (number of reducing groups/unit of volume) may vary in the different structures.
5. Treatment subsequent to the reduction of the metal may affect various structures differently.

Wolman (1955), on the basis of his experiments with specific solvents and the blocking agents prior to the impregnation of axons by the Bielschowsky procedure, suggests that the impregnation of axons is partly due to a lipid constituent and partly because of sulphhydryl and carbonyl groups, located in the axoplasm. Ethylenic linkages and hydroxyl groups, according to him, do not seem to play an important role in the process.

Peters (1955c) suggests that during impregnation, essentially, there are two processes taking place, a rapid combination of unreduced silver with the histidine and other amino acids of the tissue, and a slower formation of nuclei of reduced silver. Possibilities, that the aldehyde groups may be responsible for reduction of silver during nuclei formation, are indicated.

Winkelmann and Schmit (1959) reported that protein-bound amine groups are essential for the staining capacity of a tissue. They also believe that the histological specificity may be due to some subsequent chemical reaction rather than to the specific affinity of nerve proteins for silver ion. They have found that a water soluble aldehyde is essential as a fixative prior to the actual exposure of the tissue to silver salts, however its role is not known with certainty.

Recently Moore (1965) has described a unique protein, S-100 protein, in all parts of the nervous system of vertebrates, which, according to him, has not been found in any other tissue, and contains 1 - SH/11000 molecular weight. It may be possible that the nerve elements of Cordylophora also contain some specific protein like this which may be responsible for their differential staining with silver under the specific conditions.

The structures stained most densely with silver in the present study are the nerve cell nuclei, nucleoli, sensory hair, nerve cell processes and the outer surface of the cnidocil. The cytoplasm of the nerve cells has very little silver deposition, however, in light microscope preparations it looks much darker because of its compactness as compared with highly vacuolated cytoplasm of the ectodermal epithelio-muscular cells. Considering the very heavy deposition of silver in the nerve cell nuclei, and nucleoli in general, it seems likely that some reactive groups in the nucleic acids must be responsible for the reduction of the silver; however, it is not known whether the intense reaction reflects a mere abundance of chromatin or whether it is due to a special chemical composition of nerve cell chromatin.

The nature of the reactive groups responsible for heavy deposition of silver in the nerve cell processes, sensory hairs and the cnidocils is still unknown. According to Sleight (1962) cilia and flagella usually consist predominantly of protein, a small proportion of nucleotide material mainly as ribonucleoprotein,

and a small amount of carbohydrate material. But it is possible that since sensory hairs and cnidocils are both specialized modified cilia, their chemical nature may be different from that of true motile cilia.

The study of whole mount preparations proved to be very useful for obtaining a complete general picture of the nerve elements in Cordylophora, and the study of sections with the light and electron microscopes revealed their relative position, structure and their relation with the other tissue elements. The size, shapes, position and the presence of the characteristic sensory hairs and the nerve processes in the nerve cells were the main criteria employed to identify the nerve cells.

To recapitulate, the distinguishing features of the nerve cells in Cordylophora are:

1. the nerve cells stain specifically with the silver staining technique;
2. nerve cells are considerably smaller in size as compared to the epithelio-muscular cells of the ectoderm and have one to four characteristic long nerve processes per cell;
3. most of the nerve cells are characterized by the presence of sensory hair which may be subepithelial or projecting externally; if projecting externally, it may be carried on the tip of a long process or the nerve cell body itself;

4. the sensory hair has a typical ciliary structure arising from the base of a pit, with a root apparatus consisting of a basal body and a banded rootlet;
5. nerve cell nucleus is small, usually contains a nucleolus and lacks the fibillar and the protein bodies found in the ectodermal nuclei;
6. in addition to the mitochondria and Golgi complex, cytoplasm of the nerve cells contains three types of vesicles: the vesicles containing a dense granule in the centre (A), empty vesicles (B), and vesicles containing dense but homogeneous material (C); the A type vesicles seem to be characteristic of the nerve cells; type B vesicles are not restricted to the nerve cells alone, but are characteristically arranged beside the limiting membrane in these cells.

The nerve elements in Cordylophora have always been found in the ectoderm only. In case of Hydra some of the earlier workers (Schneider, 1890; Semal-Van Gansen, 1952) have described sparsely distributed nerve cells in the endoderm. Recently, however, Burnett and Diehl (1964) were unable to detect any nerve cells in the endoderm of Hydra. Lentz and Barrnett (1965) in their electron microscope study of the nerve cells of Hydra also report the rare occurrence of nerve cells in the endoderm. Kepner and Hopkins (1924) have shown that the endodermal muscular activity is relatively much less affected by the presence of nervous poisons like chloretone than the ectodermal system, i.e. they have shown that Hydra can

elongate normally if kept in a solution of chloretone (due to the contraction of transverse endodermal muscles) but cannot contract. Thus they suggest that the endoderm of Hydra is a neuroid tissue, as earlier suggested by Hadzi (1909). Recent work on siphonophores (Mackie, 1965) gives clear evidence of propagated depolarization in non-nervous epithelia. Considering all these evidences it seems quite conceivable that, as in Cordylophora, there are no nerve cells in the endoderm of Hydra. The earlier workers who described them, have usually used maceration techniques and it is possible that the nerve cells they described in the endoderm did not really belong there but were introduced secondarily during the dissection process as Hadzi (1909) himself has suggested. Nerves have also been found to be lacking in the endoderm of some other hydrozoans, e.g. Physalia (Mackie, 1960a). So the absence of nerve cells in the endoderm of Cordylophora is not very surprising. In the anthozoa by contrast a well developed nerve net has often been found in the endoderm (Batham, 1965; Robson, 1965).

In osmium-fixed electron microscope preparations of Cordylophora nerve cells could not be confused with interstitial cells, because interstitial cells have never been seen in this hydroid in the hydranth wall by light or by electron microscopy. Tessenow (1959) describes the occurrence of numerous interstitial cells in Cordylophora in the stem below the hydranth, and fewer in the hydranth itself; however, he did claim to have observed interstitial cells here and there in the hydranth and tentacles. It seems that what he describes as interstitial cells in the hydranth

and tentacles must have been nerve cells the processes of which he could not detect. Moreover, the fact that he does not mention the existence of nerve cells in the hydranth, makes this assumption even more likely. Moore (1952) found that there were no interstitial cells in the basal hydranth regions and the tentacles of the intact hydranths of Cordylophora. However, the accumulations of interstitial cells in the endoderm of the oral cone, described by her, were not seen in the present study.

During this study, groups of differentiating cnidoblasts have been seen in the stalk, at the base of the hydranth (Fig. 43), which suggests that the differentiation of interstitial cells takes place in the basal stalk of the hydranth, rather than in the hydranth itself. Since only the mature hydranths were studied, the origin and differentiation of the nerve cells in Cordylophora remains unknown. To establish this an extension of this study to the developing buds is required.

In spite of the physiological evidence of its ability to conduct impulses (Josephson, 1961) and contrary to Mackie's (1961) observation, no nerve cells have been found in the stolon of Cordylophora. It seems likely that the nerve cells exist only in the hydranths and not in the stolon, and if so, the conduction in the latter would be non-nervous, as suggested for the endoderm.

A certain type of desmosome possibly associated with electrical transmission from one cell to another in non-nervous tissue as suggested by Mackie (1965) have been seen in the stolon of Cordylophora

(Overton, 1963). Similar desmosomes have also been seen connecting the ectodermal cells with each other (Fig. 50) and sometimes with the nerve cells (Fig. 32) in the hydranth wall and the tentacles. Desmosomes, close apposition or apparent fusion of pre- and post-synaptic membranes have been demonstrated in synapses in the nervous tissues of various vertebrates and invertebrates in some of which the transmission is known to be electrical (Hama, 1959, 1961; Robertson, 1963; Robertson et al., 1963; Bennett, Aljure, Nakajima and Pappas, 1963; Gray, 1961; Colonnier and Guillery, 1964; Takahashi and Hama, 1965; and Yamamoto et al., 1965). However, the role of these ultrastructural features associated with electrically-transmitting junctions (synapses) is still controversial (Reger, 1965; Locke, 1965).

Categorization of the nerve cells comparable to that of Hydra according to their shape, location and presence or absence of the sensory hair (Hadzi, 1909) or ultrastructure (Lentz and Barrnett (1965) does not seem to be possible in the case of Cordylophora. In the present electron microscope study the nerve cells do show slight variations in their cytoplasmic inclusions but they are not prominent enough to be the basis for distinct classification of the nerve cells. Moreover, the differences in their physiological activity may account for some of these variations.

Regarding the role of the nervous system in the excitation of the cnidocytes and discharge of the nematocysts, there has been a conflict of opinion in the past, as to whether the cnidocytes are

independent effectors or dependent on the nervous control. According to the hypothesis of independent effectors, the cnidocil functions as the receptor as suggested by Jones (1947), Burnett, Lentz and Warren (1960) in the case of Hydra, and Westfall (1965) in Metridium. It has been found that chemical substances, like food extracts, lower the threshold of the cnidocytes to the mechanical stimuli, which then cause the nematocyst discharge (Pantin, 1942; Jones, 1947; and Ewer, 1947). However, according to Pantin (1942) mechanical stimuli of high intensity could also cause discharge of the nematocysts. Burnett, Lentz and Warren (1960) demonstrated that hydras placed in 1% chloretone or 10% alcohol, though fully paralyzed, were able to kill large numbers of Artemia. They thus suggested that the mechanism of nematocyst discharge is in no way connected with the nervous system. Jones (1947) further supported this view by the observation that the discharge is localized.

Some of the early investigators (e.g. Chun, 1881; Lendenfeld, 1887; and Murbach, 1893) believed that the nematocyst discharge is dependent on the nervous control. Jones (1947) found that many agents eliciting discharge in the living Hydra are ineffective when the animal has been anaesthetized, indicating that the control does not lie in the cnidocil alone. Similar effects of the anaesthetics, like chloretone, chloral hydrate, and magnesium sulphate, on the nematocyst discharge in a siphonophore Physalia, have been demonstrated by Mackie (1960a). However, according to this author the strong solutions of the same chemicals had an irritant effect and caused discharge. Ross and his collaborators have shown several examples

in which nematocyst discharge appears to be functionally linked with specific behavior patterns (Ross and Sutton, 1964; Davenport, Ross and Sutton, 1961). Recently Lentz and Barrnett (1961, 1962) in their histochemical studies have demonstrated sites of cholinesterase activity apparently connecting the nerve cells and the cnidocytes. They also reported that acetylcholine produced a considerable discharge of nematocysts which was augmented by eserine, a cholinesterase inhibitor.

Most workers on hydroids agree that nerve elements are frequently in contact with cnidocytes (Spangenberg and Ham, 1960; Lentz and Barrnett, 1965), if not actually terminating on them. This has been confirmed in the present study. There is clearly ample opportunity for the nerves to exercise control over the cnidocyte function, if all that is required, is frequency of contact between the two. It is also to be noted that epithelial cells are in contact with cnidocytes. Since the former may show conduction phenomena (Mackie, 1965), it is not impossible that cnidocyte function could be influenced by electrical events in these cells quite apart from the influence of the nerve cells.

V. SUMMARY

1. The nerve elements in a hydroid Cordylophora lacustris were studied with light and electron microscopes. A modified Peters' silver method was used for staining the nerve cells and their processes specifically. The nerve elements were identified in the osmium fixed electron microscope preparations by their shapes, location and certain other characters corresponding to those of the nerve cells seen in silver stained light and electron microscope preparations. Study of the silver stained preparations with the electron microscope proved to be effective in establishing a correlation between the light and electron microscopic observations.
2. The nerve cells are found only in the ectodermal layer, where they are located intra- or subepithelially. Local concentrations of nerve elements comparable to those described in Hydra have not been found in Cordylophora. In the hydranth, the number of nerve cells varies from 4 to 7 per $(100\mu)^2$.
3. The nerve cells and their processes are found in all the major regions of the hydranth and tentacles, but not in the stolon.
4. The nerve cells are much smaller than the epithelio-muscular cells and their cytoplasm is less vacuolated. They are distinguishable from other cellular elements by the presence of their characteristic long and often branched processes.

The first thing I noticed when I stepped out of the car was the cold. It was a sharp contrast to the warm blanket I had been sitting under. I looked up at the sky, which was a deep, dark blue, and felt a sense of peace. The air was crisp and clean, and I could hear the distant sounds of the city. I took a deep breath and felt a sense of renewal. I had been so stressed and overwhelmed, but now I felt like I was starting over. I looked down at my hands, which were slightly numb from the cold, and felt a sense of hope. I knew that I was going to make it through this. I was going to be a doctor. I was going to be a doctor.

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5. Nerve processes in whole mount preparations are found to be orientated more or less longitudinally. Their total visible length ranges from 100 to 200 μ per nerve cell body. The thickness varies from roughly 1000 m μ near the nerve cell bodies to less than 100 m μ in case of finer branches.
6. The shapes of the nerve cells and the number of processes per nerve cell body vary. The relative abundance of mono-, bi-, tri- and tetrapolar nerve cells is 8%, 70%, 17% and 5% respectively.
7. Most of the nerve cells carry a characteristic short sensory hair which may be subepithelial or projecting externally; in the latter case it can be carried at the tip of a long process or spring from the nerve cell body itself.
8. The sensory hair stains very densely with silver, and arises from the base of a pit, 600 - 1000 m μ wide. It is roughly 250 - 400 m μ thick and is never found to be longer than 3 μ . In osmium-fixed electron microscope preparations, it shows a typical 9 + 2 ciliary structure. The nine peripheral tubules merge at the base into a basal body. The lower part of the basal body, the centriole, is associated with a banded rootlet, which in one case has been found to be more than 3 μ long reaching almost half the length of the nerve cell itself. Occasionally a second centriole, in cross section, has been found in association with the root apparatus.

9. The sensory hair of a nerve cell is distinguishable from the cnidocil by the absence of the supporting structures around its base. Moreover, the cnidocil has a dense core instead of the central tubules, found in the sensory hair.
10. The nuclei of the nerve cells are smaller and more densely stained with silver than those of the epithelio-muscular cells. Nucleoli are usually present. In osmium preparations nerve cell nuclei are easily distinguishable from ectodermal nuclei by the absence of the protein and the fibrillar bodies, found frequently in the latter.
11. In addition to the mitochondria and the elaborate Golgi complex, nerve cells often contain vesicles which can be classed into three types:- Type-A vesicles, characterized by the presence of electron dense granules in their central areas. They vary roughly from 100 to 250 μ in diameter and can be found occasionally in the Golgi area. Type-B vesicles which lack the electron dense granules found in type-A, and are of variable sizes. They can be roughly categorized into "small" and "large" B-type vesicles; however, occasionally some intermediary forms are observed. The diameter of the small B-type vesicles is about 100 - 200 m μ . They are more or less round in shape, and are often characteristically concentrated beside the nerve cell membranes. They have also been found in Golgi areas or scattered in the cytoplasm. Large B-type vesicles are more irregular in shape and fewer in number. Type-C, vesicles containing homogeneous, but moderately dense material, mostly associated with the Golgi complex.

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12. All the three types of vesicles can occasionally be found in a single nerve cell. Type-A vesicles were not seen in epithelio-muscular cells or in the cnidocytes. Type-B vesicles are not restricted to nerve cells alone; however, their regular arrangement beside the cell membrane is characteristic of the nerve cells.
13. Multivesicular bodies of unknown nature, lipid bodies, and lysosomes are also found in some nerve cells. Endoplasmic reticulum is not a very prominent feature of the nerve cells in Cordylophora, however, profiles resembling rough-surfaced endoplasmic reticulum are found in some examples. Occasionally free ribosomes have also been found.
14. Classification into "sensory" and "ganglion" cells on the basis of shapes, location and presence or absence of a sensory hair is not possible in the case of Cordylophora. Nor can the various nerve elements be distinguished on grounds of differing cytoplasmic structure. In osmium-fixed electron microscope preparations no clear-cut distinction such as is described for Hydra between ribosome rich, ribosome poor or neurosecretory cells seems possible.
15. Nerve processes stained very heavily with silver. In osmium preparations, they contained type-A and small type-B vesicles and occasionally a few ribosomes.

16. Occasionally nerve cells are found in groups of two to three but without showing any intimate contacts with each other. Nerve cells are often found closely associated with the epithelio-muscular cells, muscle processes and the cnidocytes. But whether their contacts are physiologically significant, is not known.

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VII. EXPLANATION OF FIGURES

Figures 1, 2 and 22 are drawings; Figure 3 is a histogram; Figures 4, 5 and 22A are light micrographs; and the rest are all electron micrographs. In all cases originals were made, labelled and then reproduced photographically. Consequently there is some loss of resolution in all the micrographs.

All the micrographs of silver stained preparations except Fig. 15, and Figs. 23, 26, 28, 33, 36, 37, 39, 41 and 42 of osmium-fixed preparations are based on sections of hydranths, more or less stretched before fixation.

Frontispiece:- A photograph of Cordylophora lacustris showing few hydranths and a portion of the stolon.

H, hydranth; st, stolon; T, tentacles; h, hypostome.

X 12.

Fig. 1. Camera lucida drawing showing a typical part of the nerve net in the hydranth wall.

c, cnidocyte; n, nerve process; ncb, nerve cell body;
ne, ectodermal nucleus; sh, sensory hair; ssh,
subepithelial sensory hair.

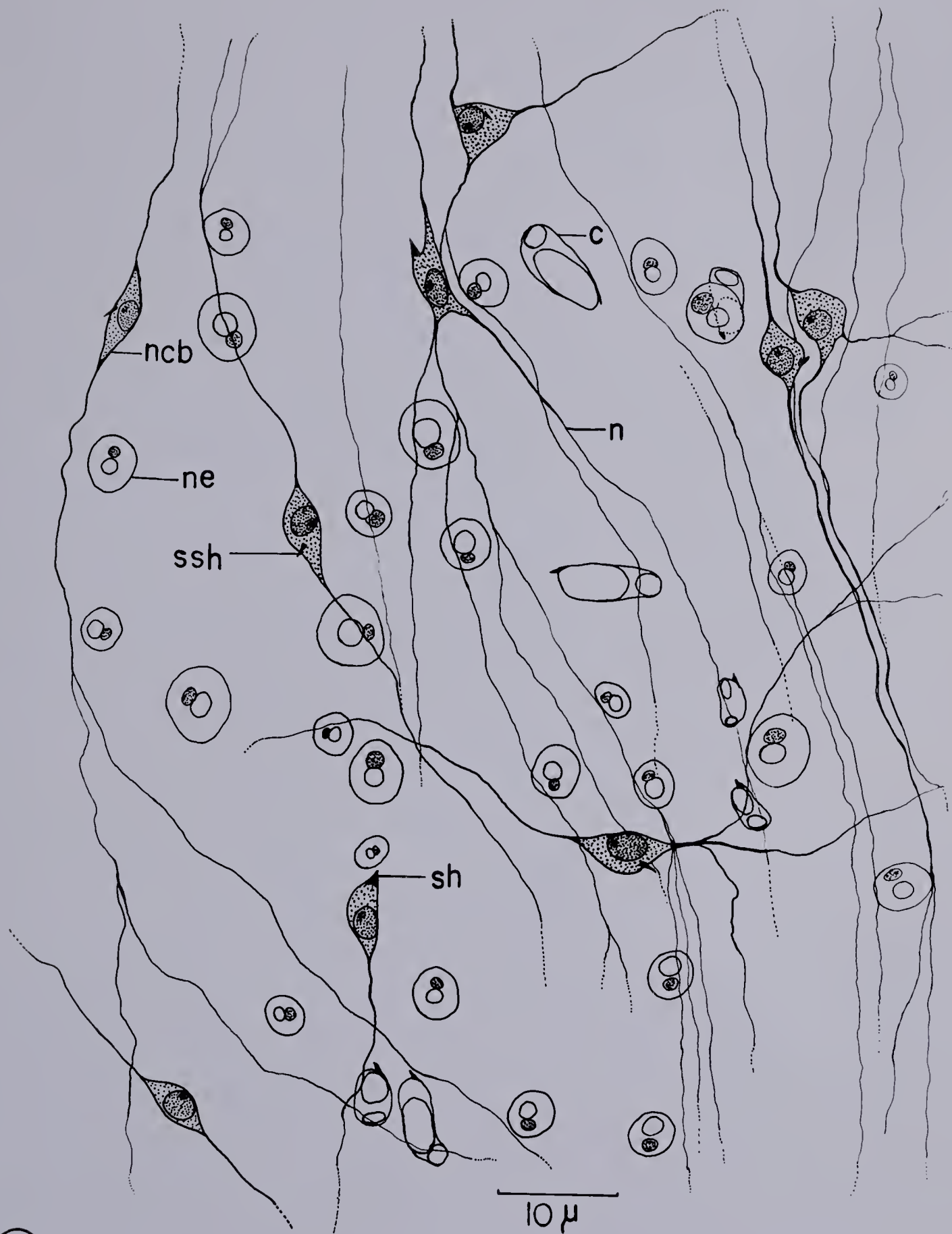


Fig. 2. A drawing of the various configurations of nerve cell bodies as seen in the light microscope study of silver stained preparations.

n, nerve process; p, process running up to the surface of the epithelium with a short sensory hair (sh) at its tip; ssh, subepithelial sensory hair.

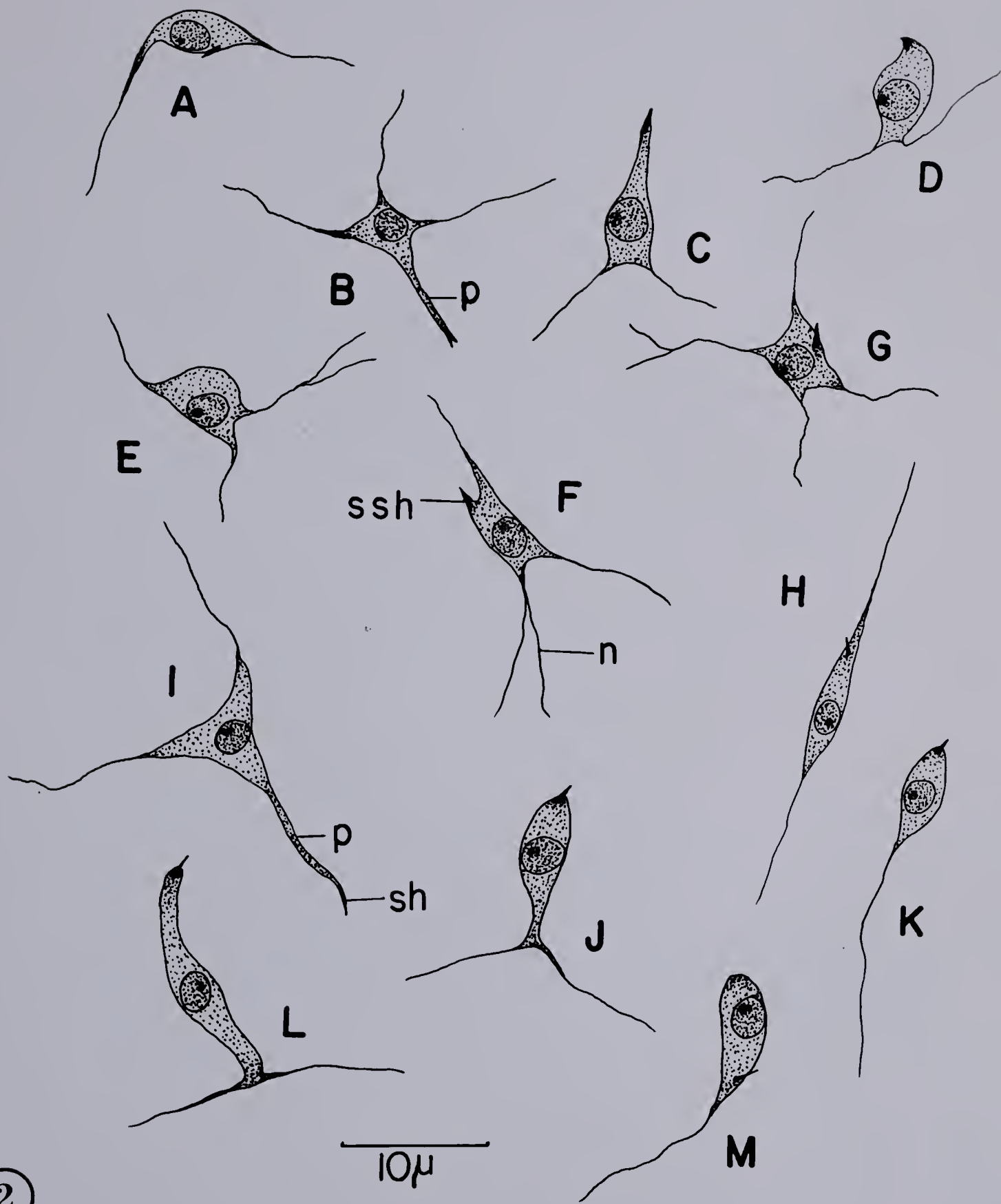


Fig. 3. A histogram showing the orientation of neurites with respect to the longitudinal axis of the hydranth wall.

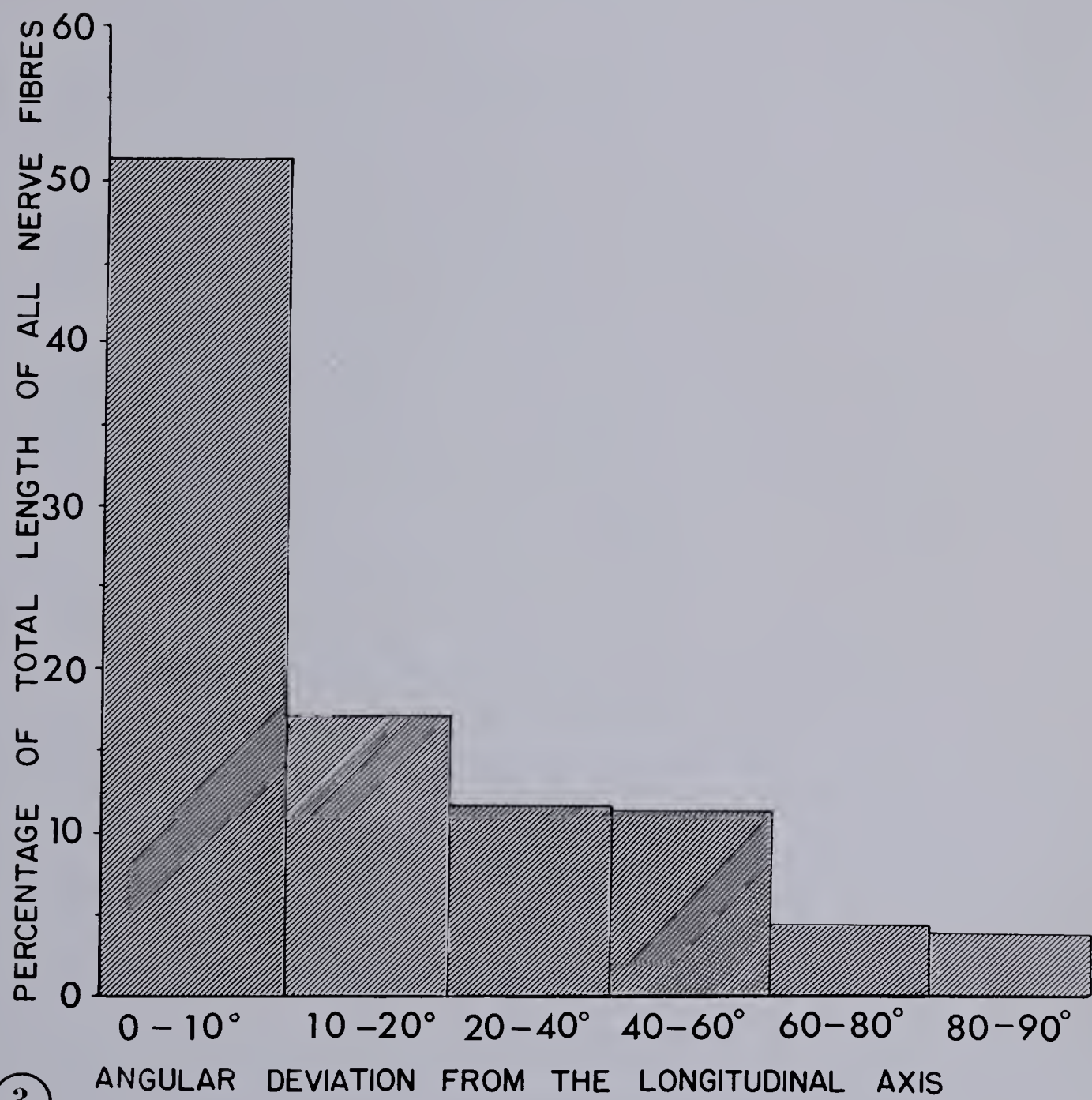


Fig. 4. Light micrographs of different kinds of nerve cells and of some areas in the hydranth wall showing the general pattern of the nervous system in the whole mount preparations of Cordylophora.

n, nerve process; N, nerve cell nucleus; ncb, nerve cell body; ne, ectodermal nucleus; P, pit; p, process running up to the surface of the epithelium with a short sensory hair (sh) at its tip; ssh, subepithelial sensory hair.

Picroformol fixation and silver stain. Whole mount preparations. Scale = 10 μ .

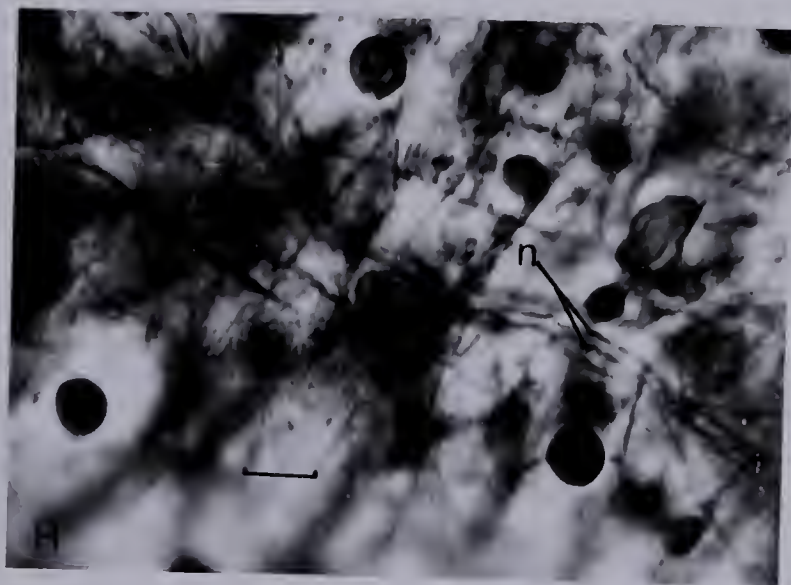
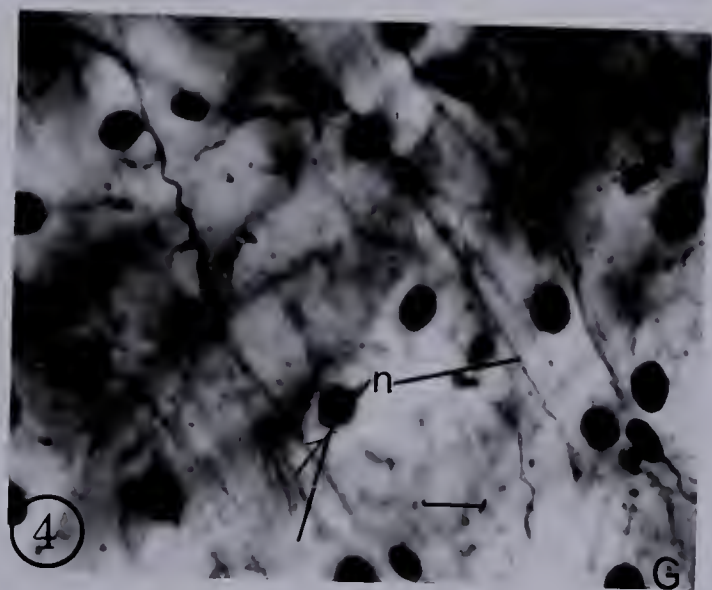
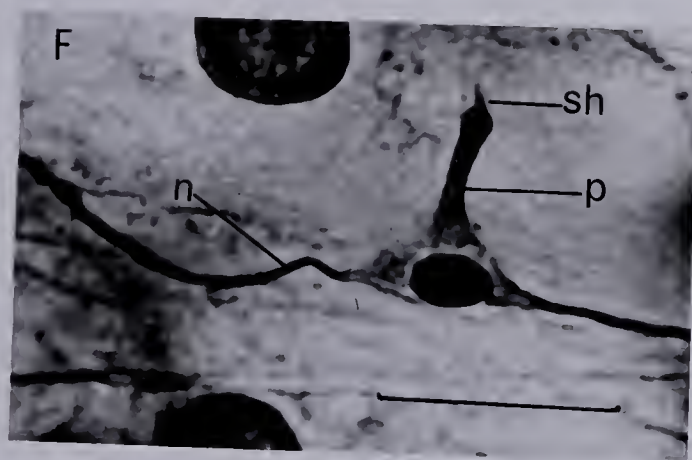
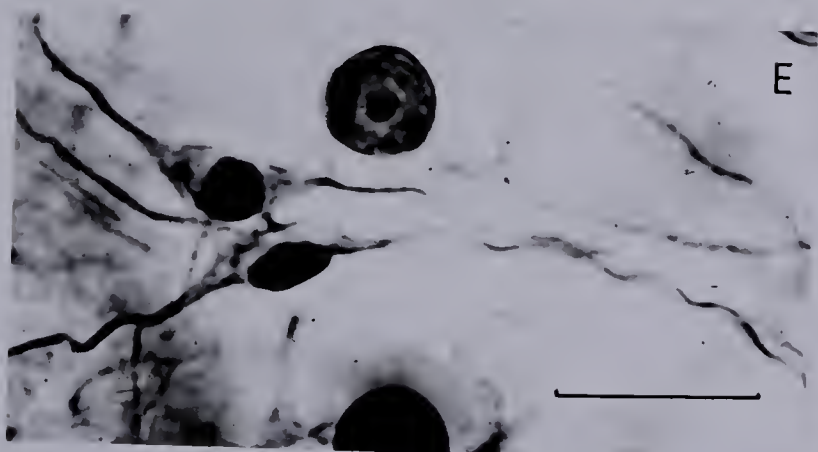
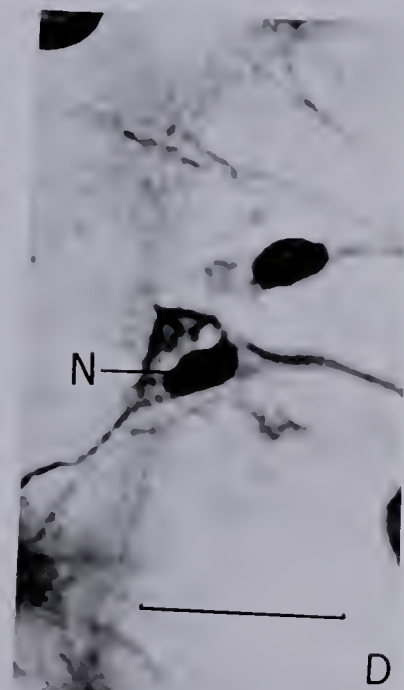
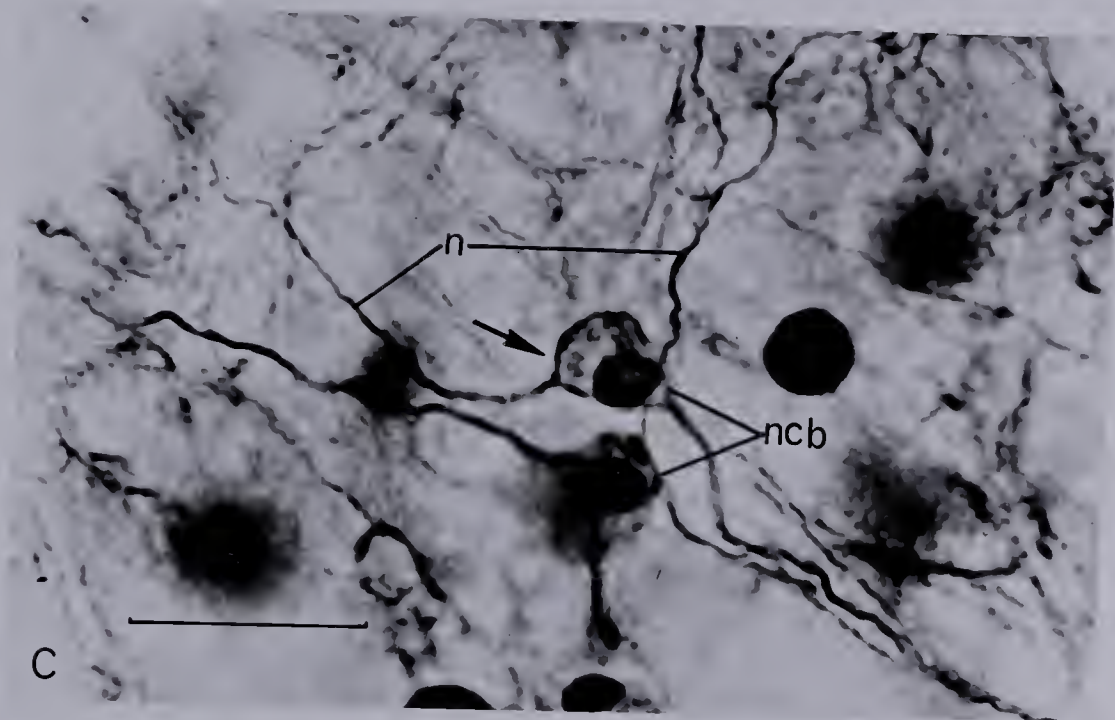
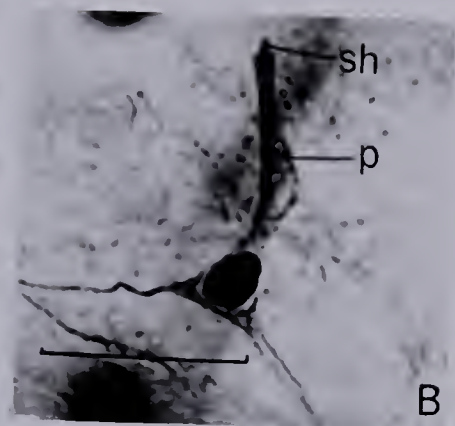
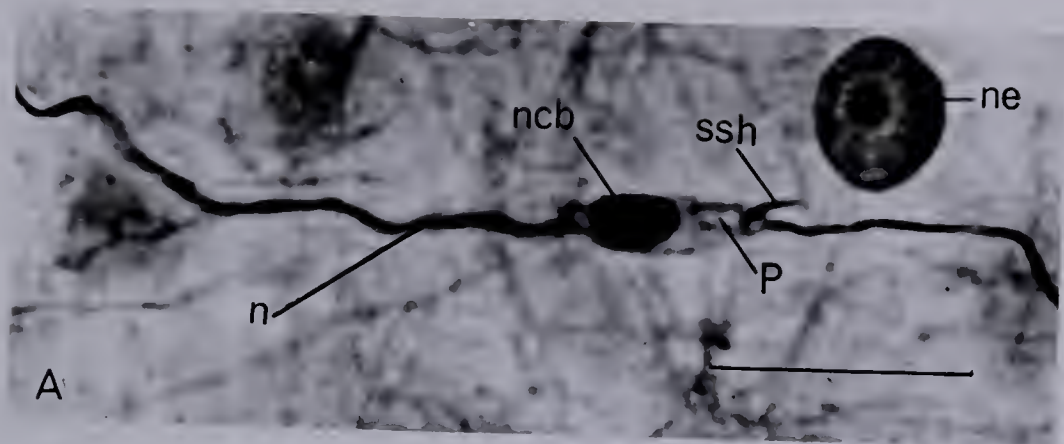


Fig. 5. Light micrographs of nerve cells of Cordylophora.

Whole mount preparations, except G which is a tangential section, 10 μ thick.

c, cnidocyte; n, nerve process; N, nerve cell nucleus; Nc, cnidocyte nucleus; ne, ectodermal nucleus; nt, nematocyst; nu, nucleolus; pb, protein body; ssh, subepithelial sensory hair.

Picroformol fixation and silver stain. Scale = 10 μ .

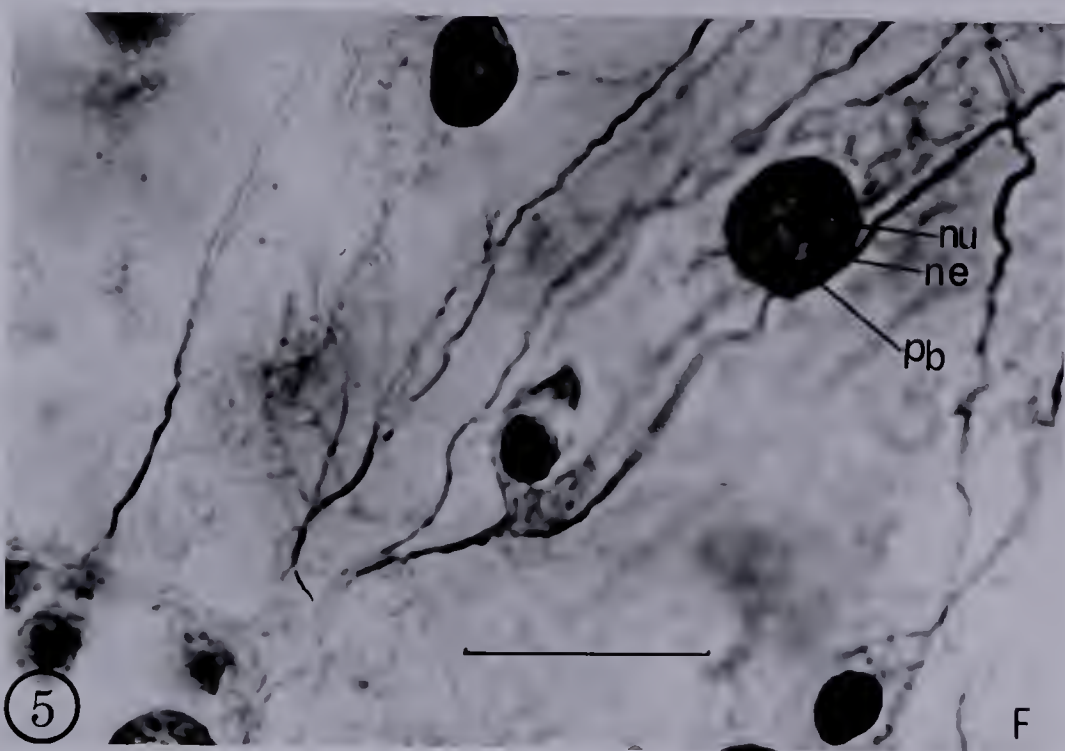
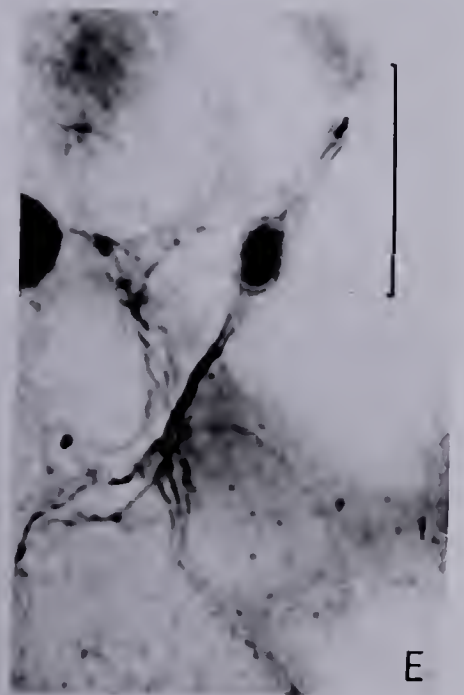
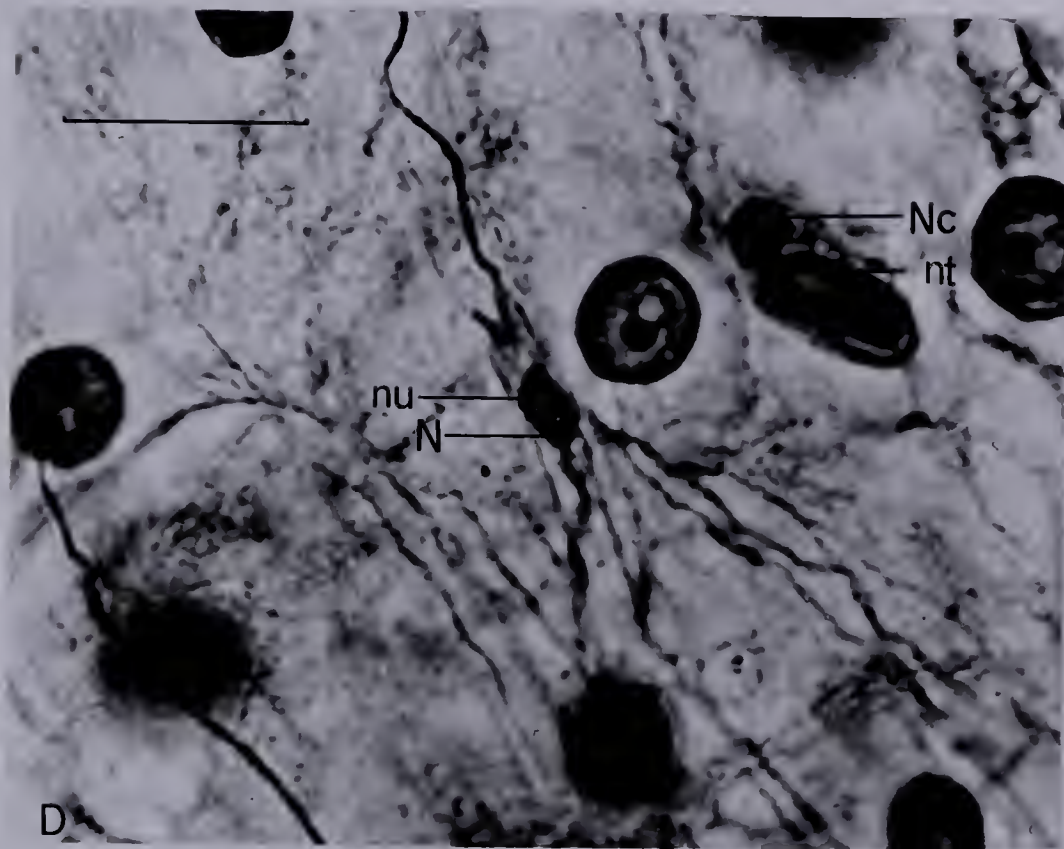
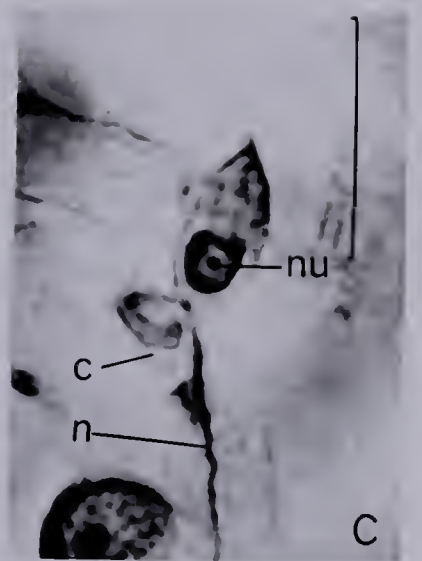
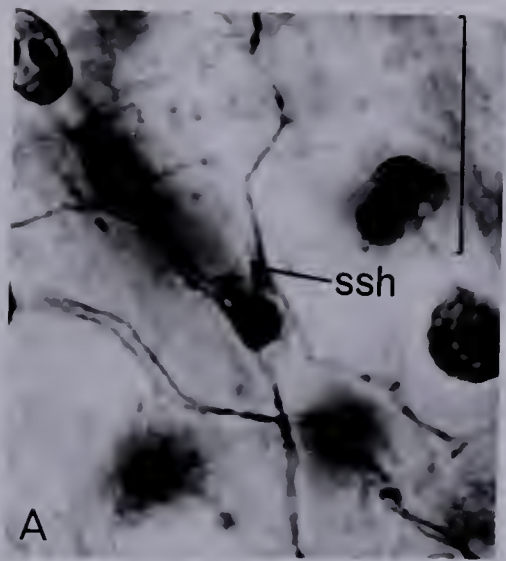


Fig. 6. Section of the hydranth wall showing a nerve cell in the ectoderm.

EC, ectoderm; EN, endoderm; ME, mesoglea; N, nerve cell nucleus; nu, nucleolus; OS, outer surface.

Picroformol fixation, silver stain, Araldite embedding, and uranyl acetate counterstaining.

X 13,000.

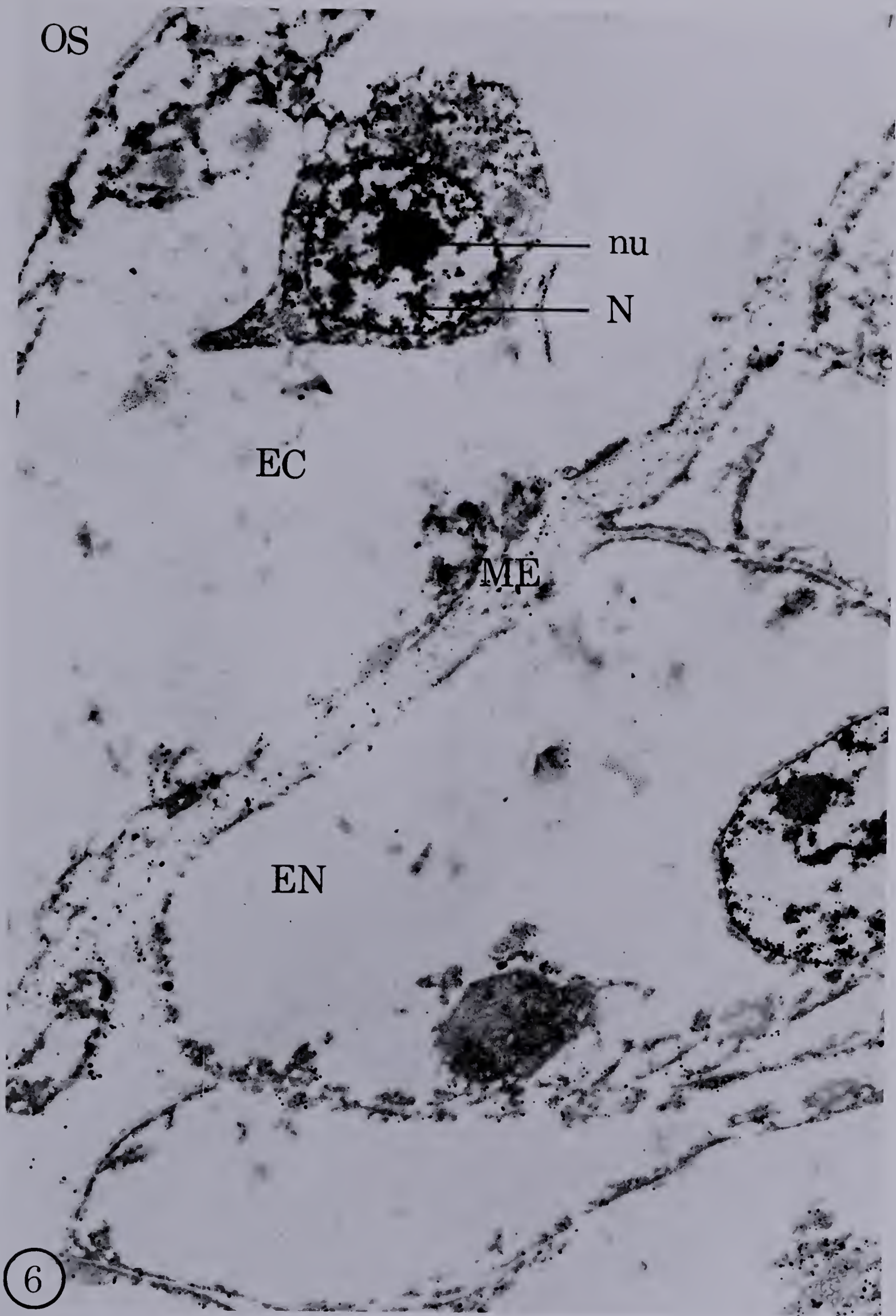


Fig. 7. Section of the hydranth wall showing a nerve cell with a subepithelial sensory hair.

EC, ectoderm; EN, endoderm; N, nerve cell nucleus;
ncb, nerve cell body; Nen, endodermal nucleus;
ssh, subepithelial sensory hair.

Picrformol fixation, silver staining, Araldite embedding,
and uranyl acetate counterstaining. X 9,000.

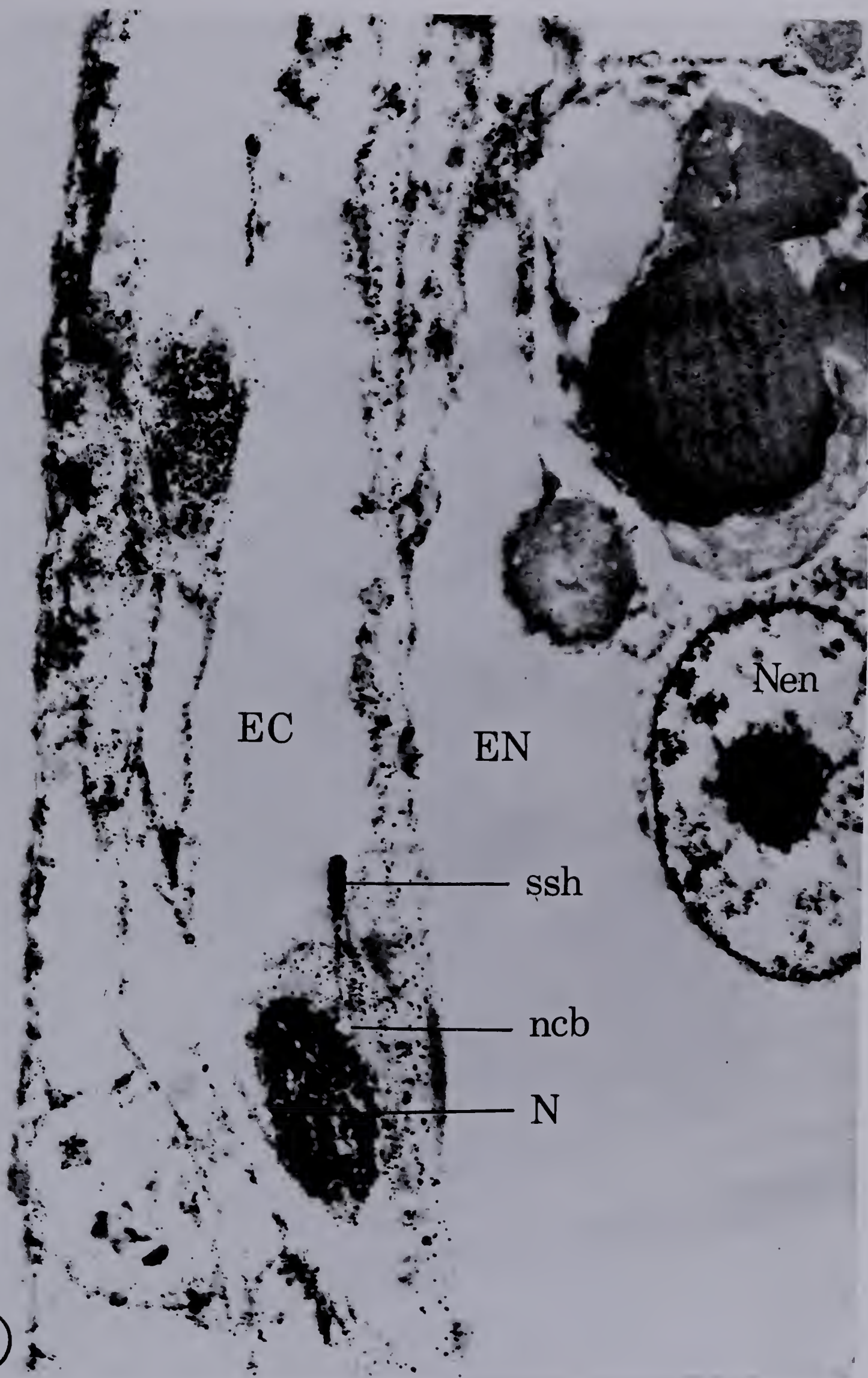


Fig. 8. Enlarged picture of the nerve cell seen in Fig. 7.
Note the heavy deposition of silver in the nucleus
and the subepithelial sensory hair. X 30,000.

ME, mesoglea; N, nerve cell nucleus; ncb, nerve cell
body; ssh, subepithelial sensory hair.

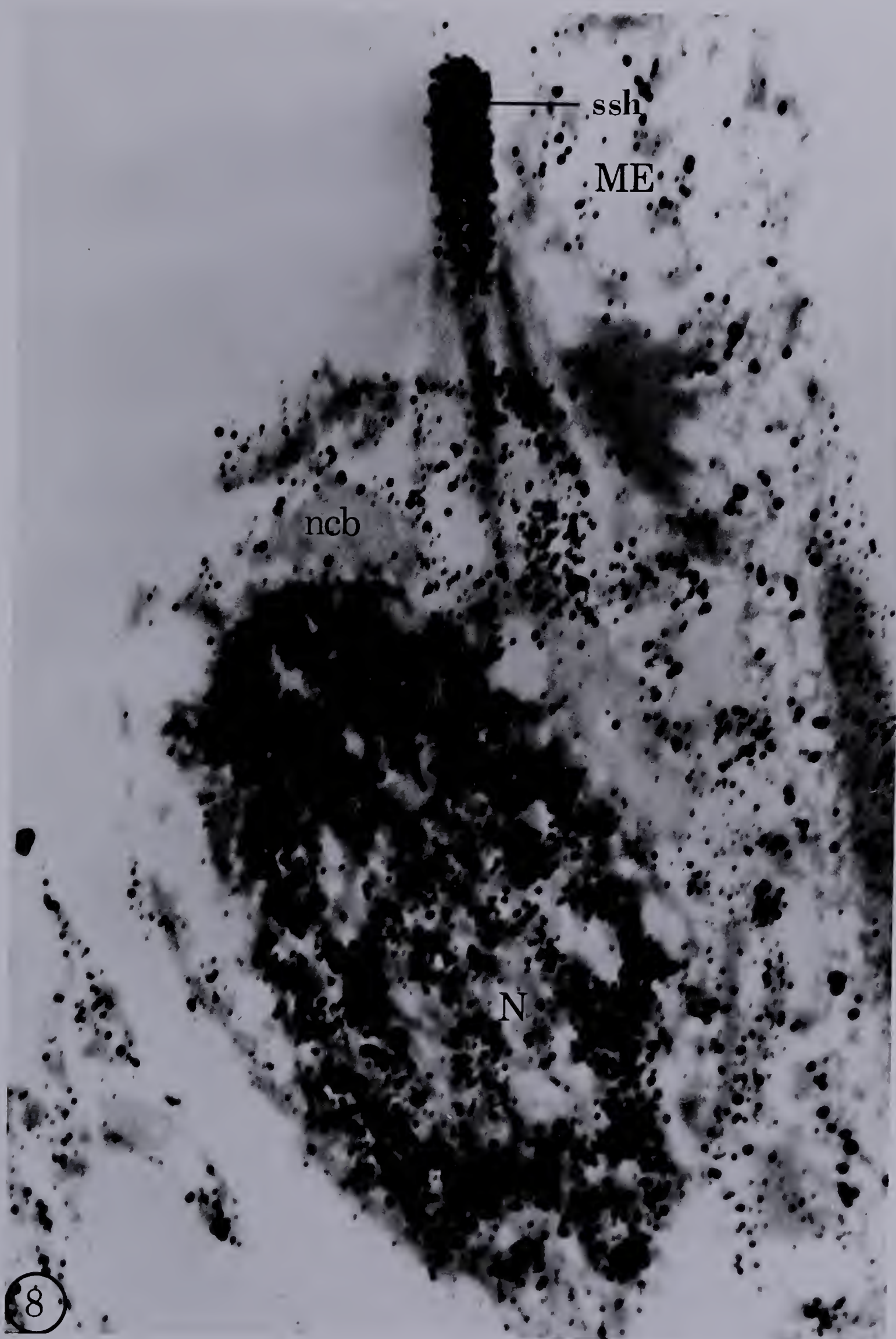


Fig. 9. A nerve cell with a subepithelial sensory hair lying in a pit. Note the close association and heavy deposition of silver (pointed by an arrow) between the nerve cell and a cnidocyte capsule containing the nematocyst.

EC, ectoderm; N, nerve cell nucleus; nt, nematocyst; OS, outer surface; P, pit; ssh, subepithelial sensory hair.

Picroformol fixation, silver stain and Araldite embedding, counterstain uranyl acetate. X 27,000.

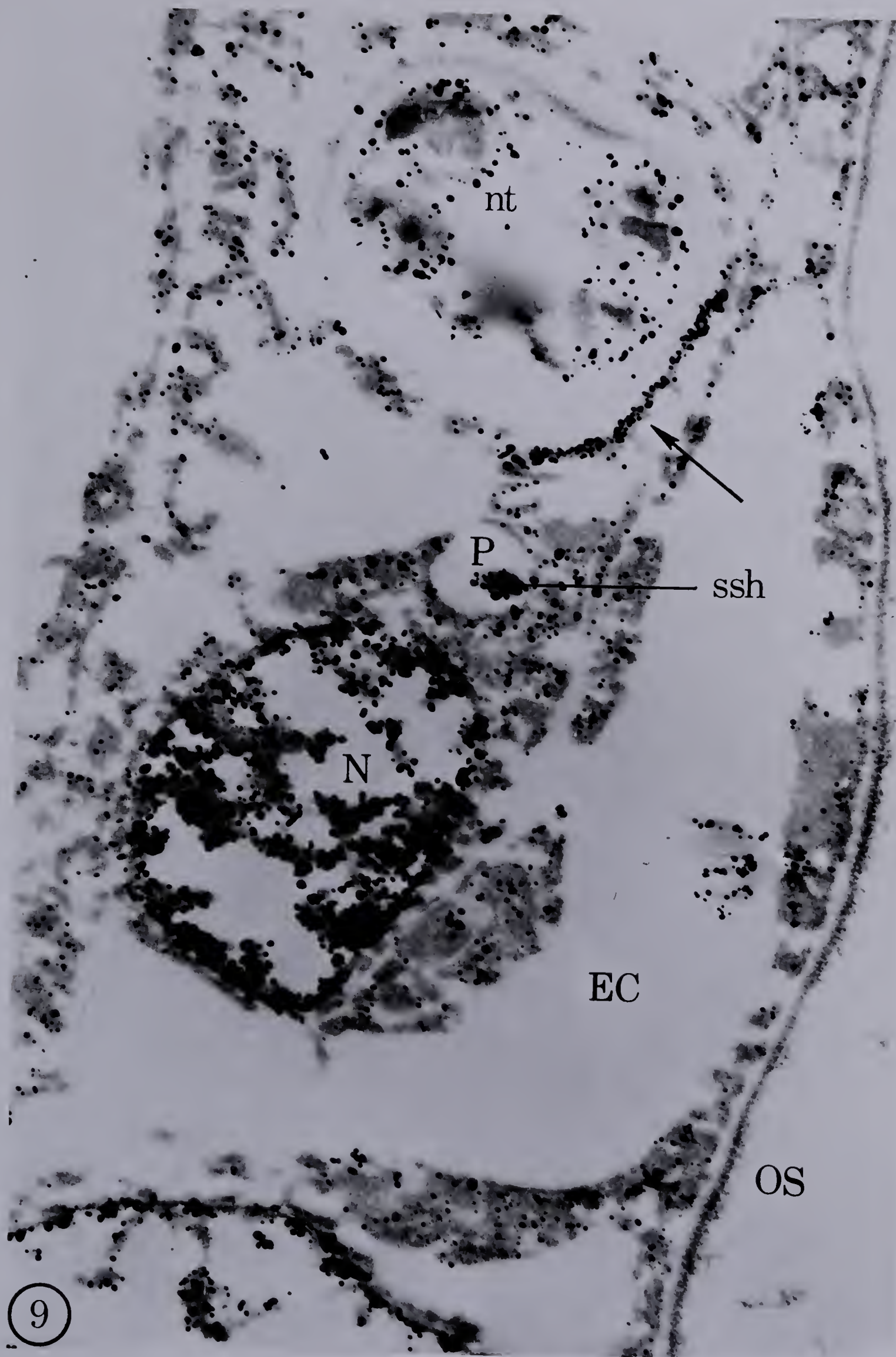


Fig. 10. A nerve cell body showing a continuation with a heavily impregnated nerve process at one end.

N, nerve cell nucleus; nu, nucleolus; n, nerve process.

Picroformol fixation, silver stain and Araldite embedding. X 25,000.

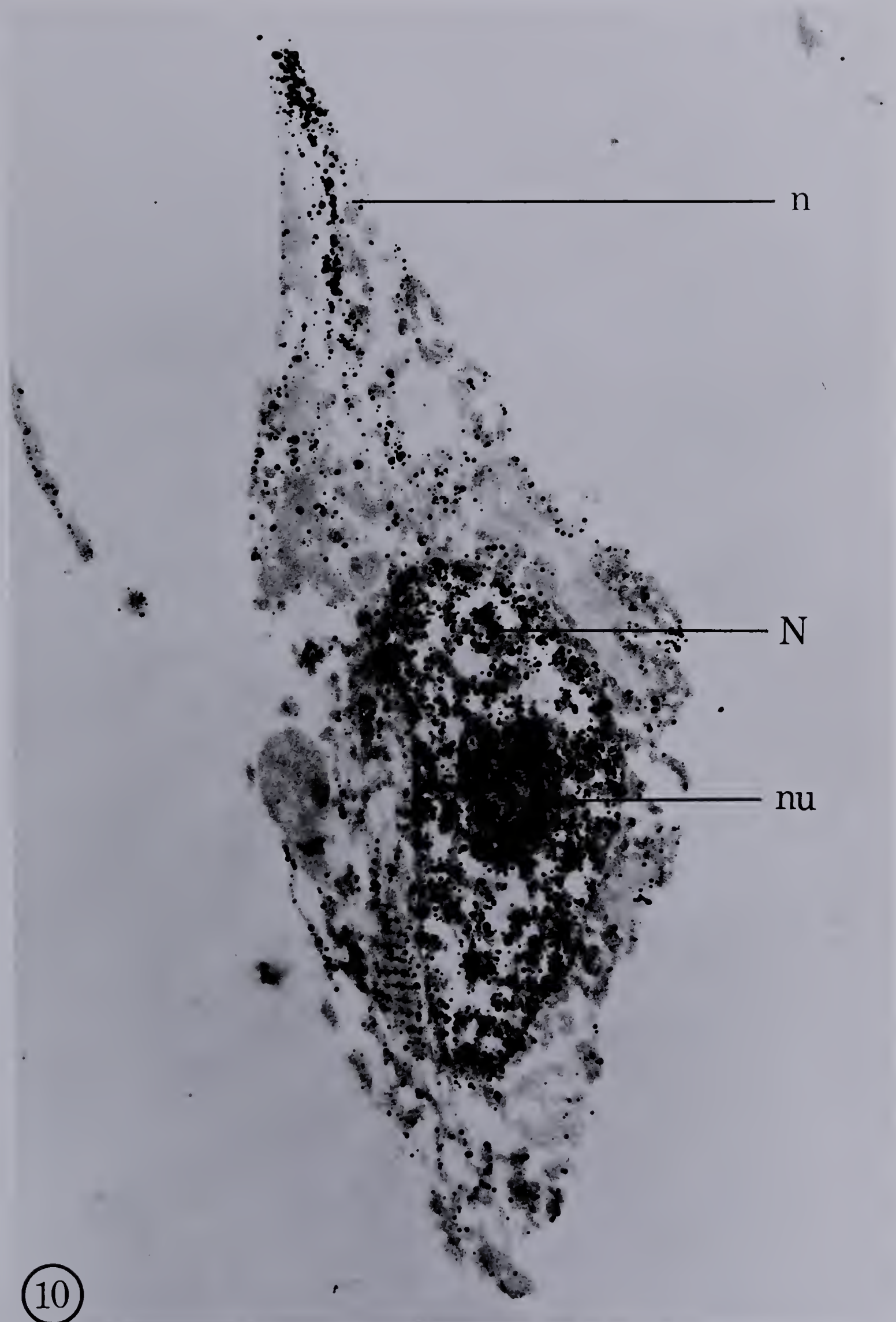


Fig. 11. A nerve cell body with two processes showing heavy deposition of silver.

N, nerve cell nucleus; n, nerve process.

Picroformol fixation, silver stain and Araldite embedding. X 25,000.

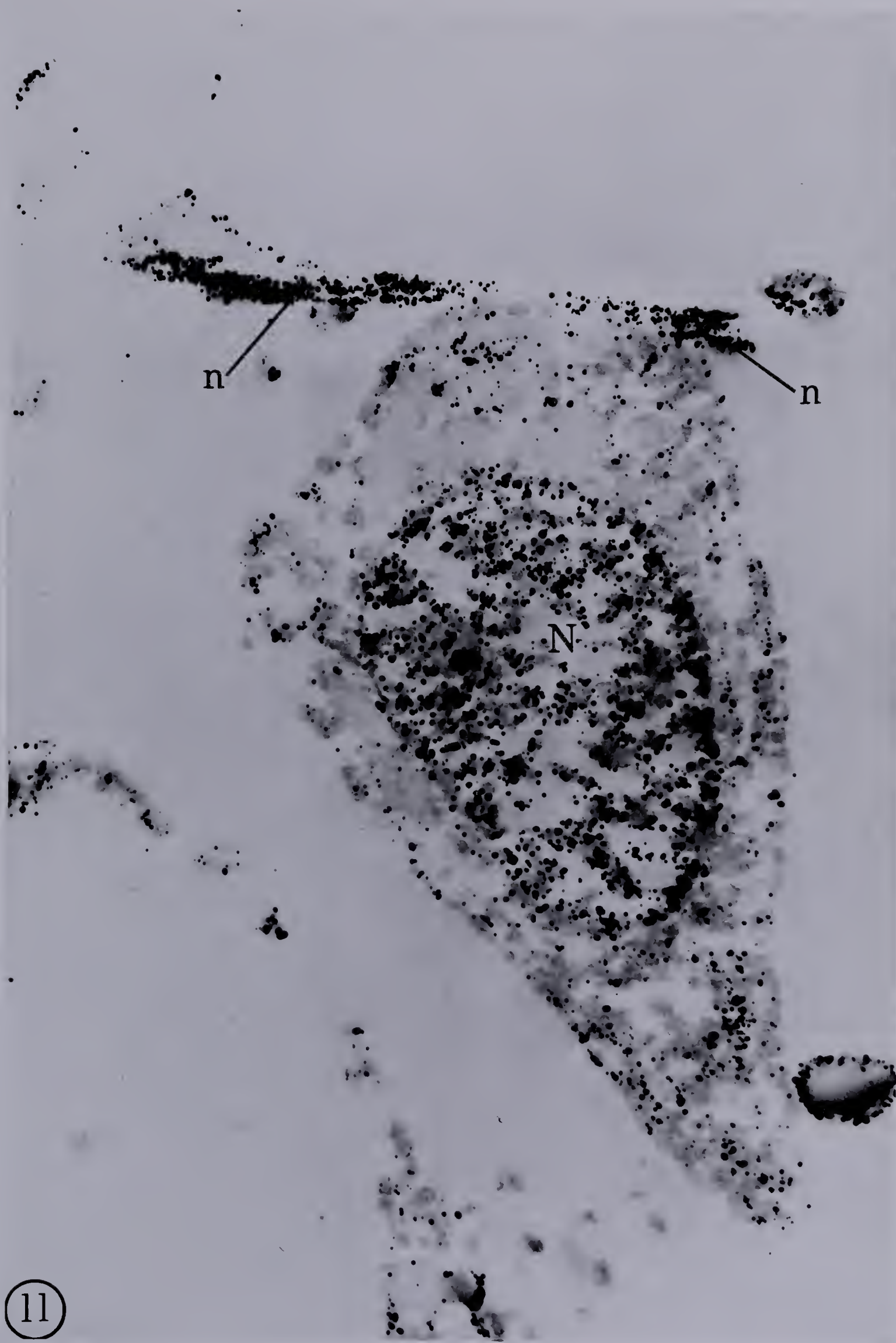


Fig. 12. A section showing a nerve cell, and pieces of free-lying, heavily impregnated nerve processes.

N, nerve cell nucleus; n, nerve process; ncb, nerve cell body.

Picroformol fixation, silver stain and Araldite embedding, counterstained with uranyl acetate.

X 20,000.

n

N

ncb

n

Figs. 13 and 14. Electron micrographs of nerve processes (n) suggesting that the chief sites of silver deposition are located mainly at the surface. Arrows point towards the central "core" regions which show little silver deposition.

Picroformol fixation, silver stain and Araldite embedding, counterstain uranyl acetate.

Fig. 13 X 20,000.

Fig. 14 X 27,000.

13



14

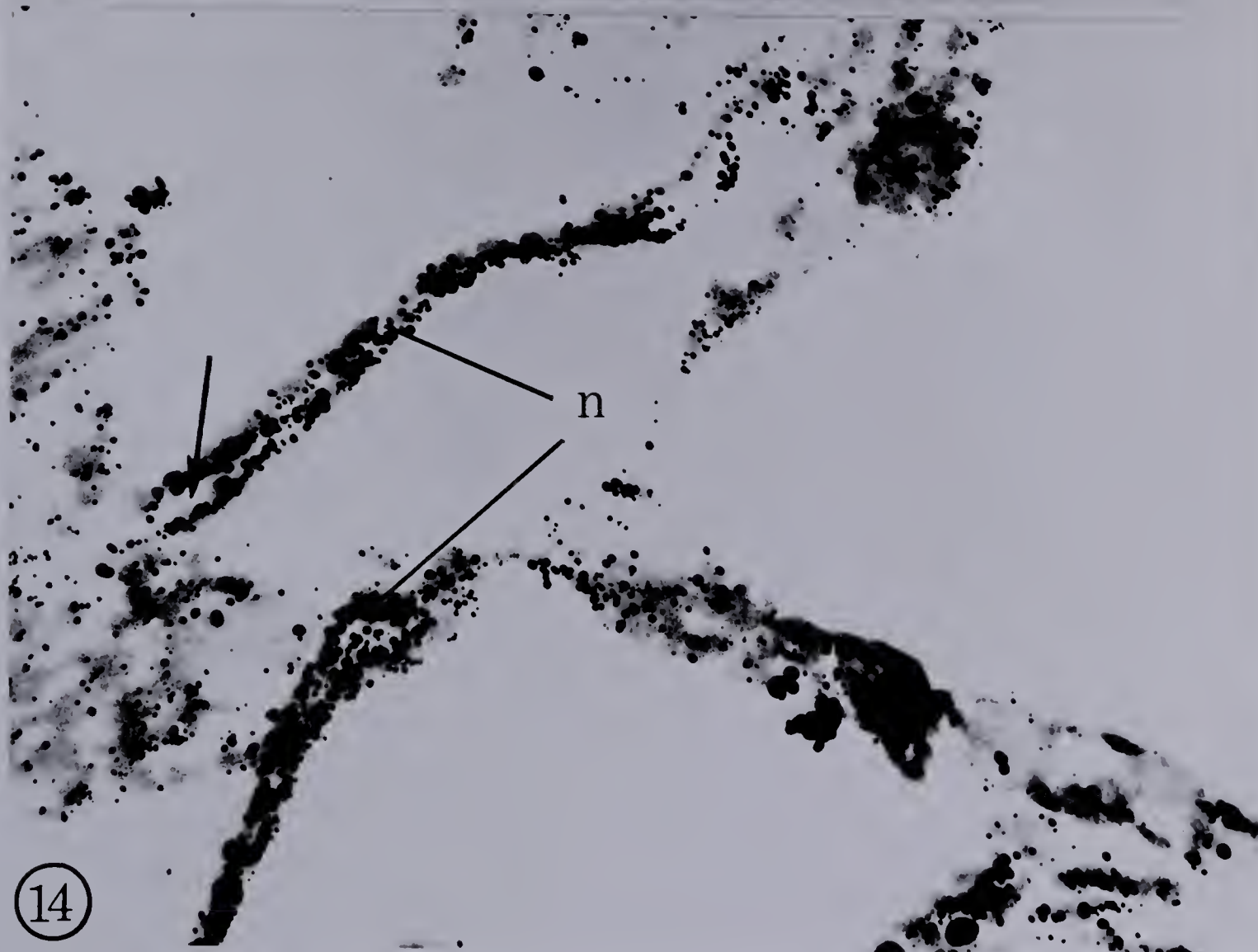


Fig. 15. A section through the ectodermal surface of a tentacle, showing a nerve cell among a group of cnidocytes.

Note and compare the density of silver in, and the size and structure of the ectodermal nucleus, the nerve cell nucleus and the cnidocyte nuclei.

N, nerve cell nucleus; ne, ectodermal nucleus;

Nc, cnidocyte nucleus; nt, nematocyst, cc, cnidocil.

Picroformol fixation, silver stain and Araldite embedding, counterstain uranyl acetate.

X 12,000.

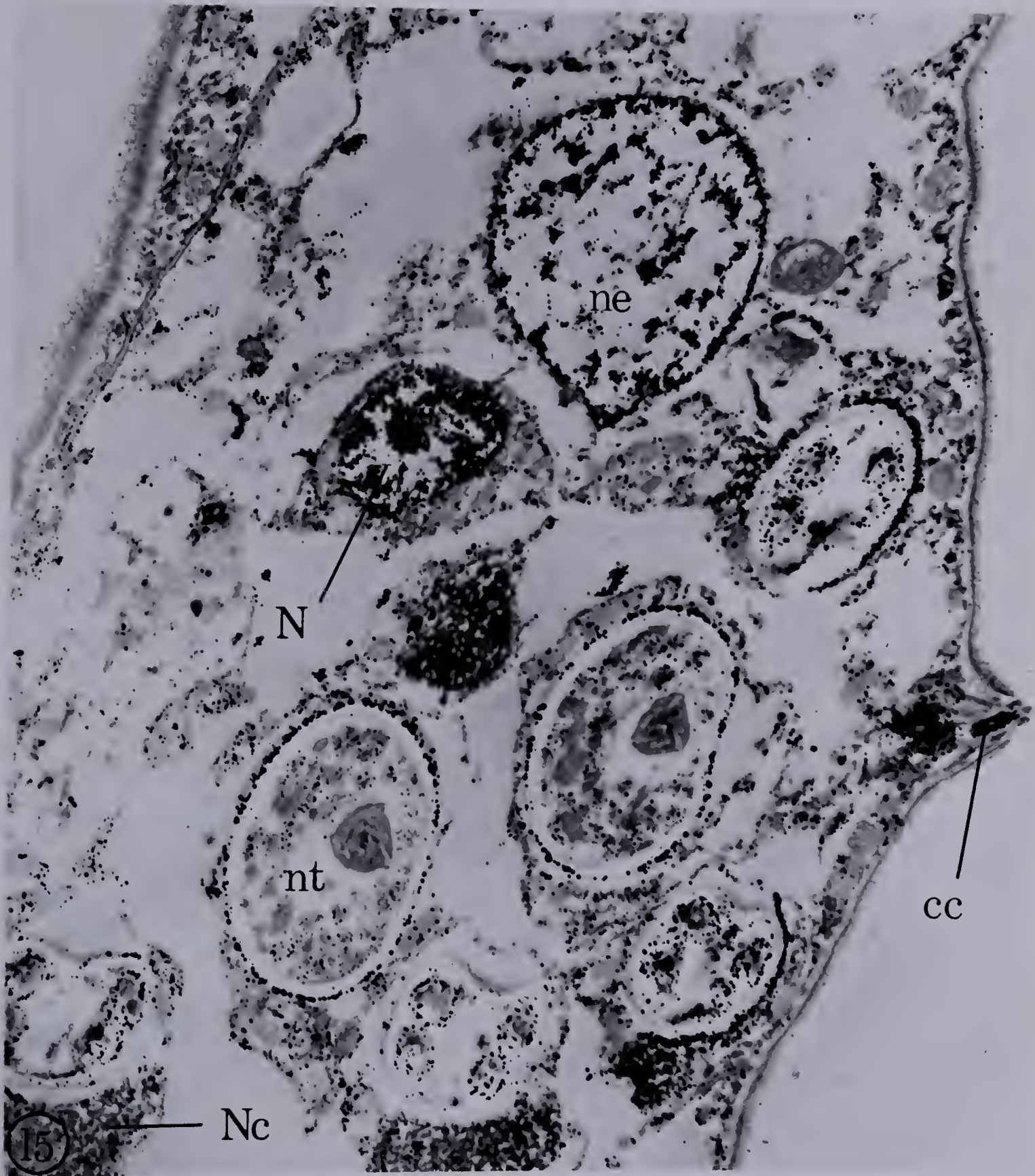


Fig. 15A. Two nerve cell bodies lying close to each other and one of them showing a continuation into a nerve process.

N, nerve cell nucleus; ncb, nerve cell body; n, nerve process.

Picroformol fixation, silver stain, Araldite embedding and uranyl acetate counterstaining.
X 27,000.

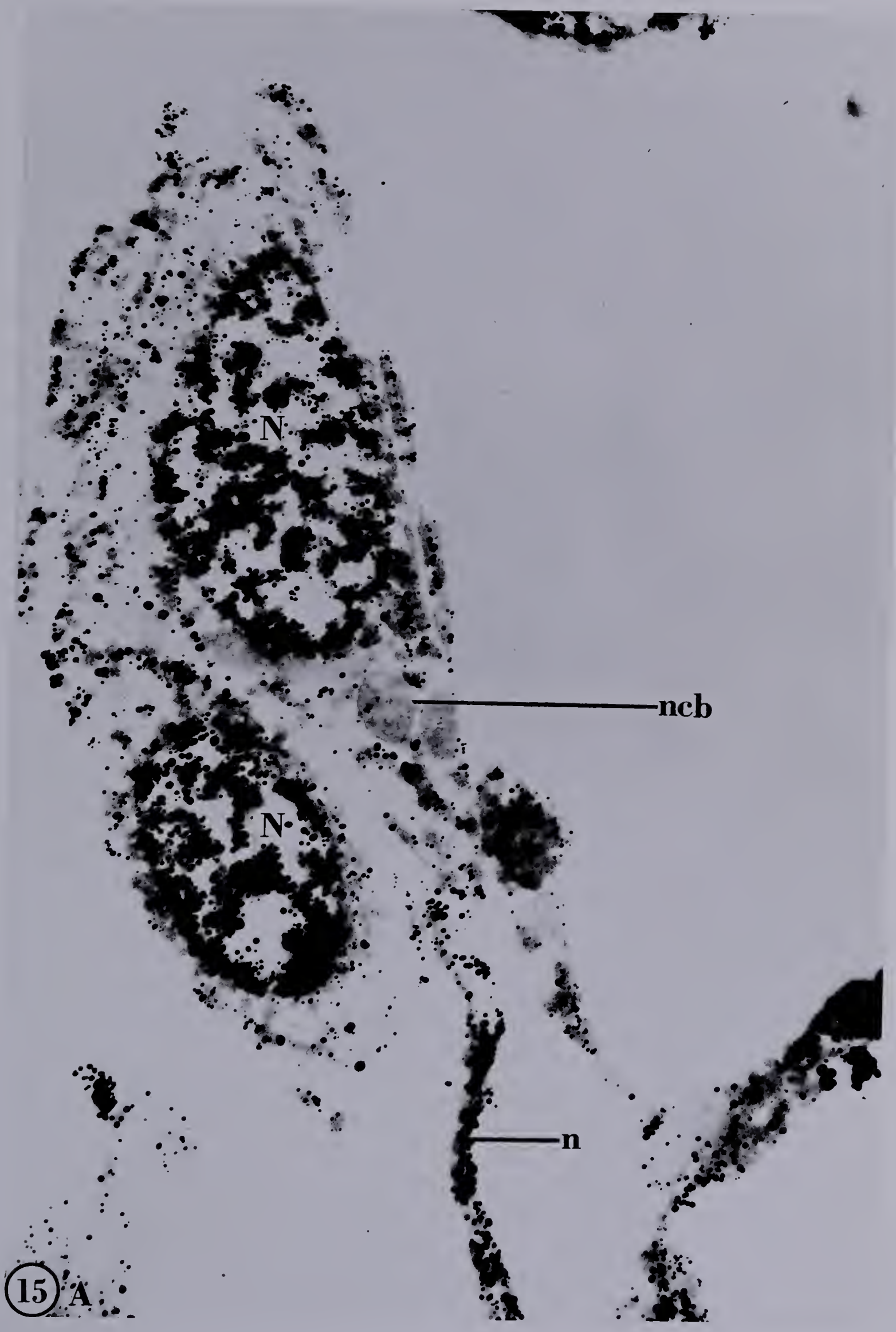


Fig. 16. An oblique section through the hydranth wall showing a nerve cell and an ectodermal nucleus. Note the difference in size and density of silver in the two nuclei, and the presence of the characteristic protein body in the ectodermal nucleus which is lacking in the nerve cell nucleus.

ncb, nerve cell body; N, nerve cell nucleus; ne, ectodermal nucleus; nu, nucleolus; pb, protein body.

Picroformol fixation, silver stain and Araldite embedding, counter stain uranyl acetate.

X 20,000.

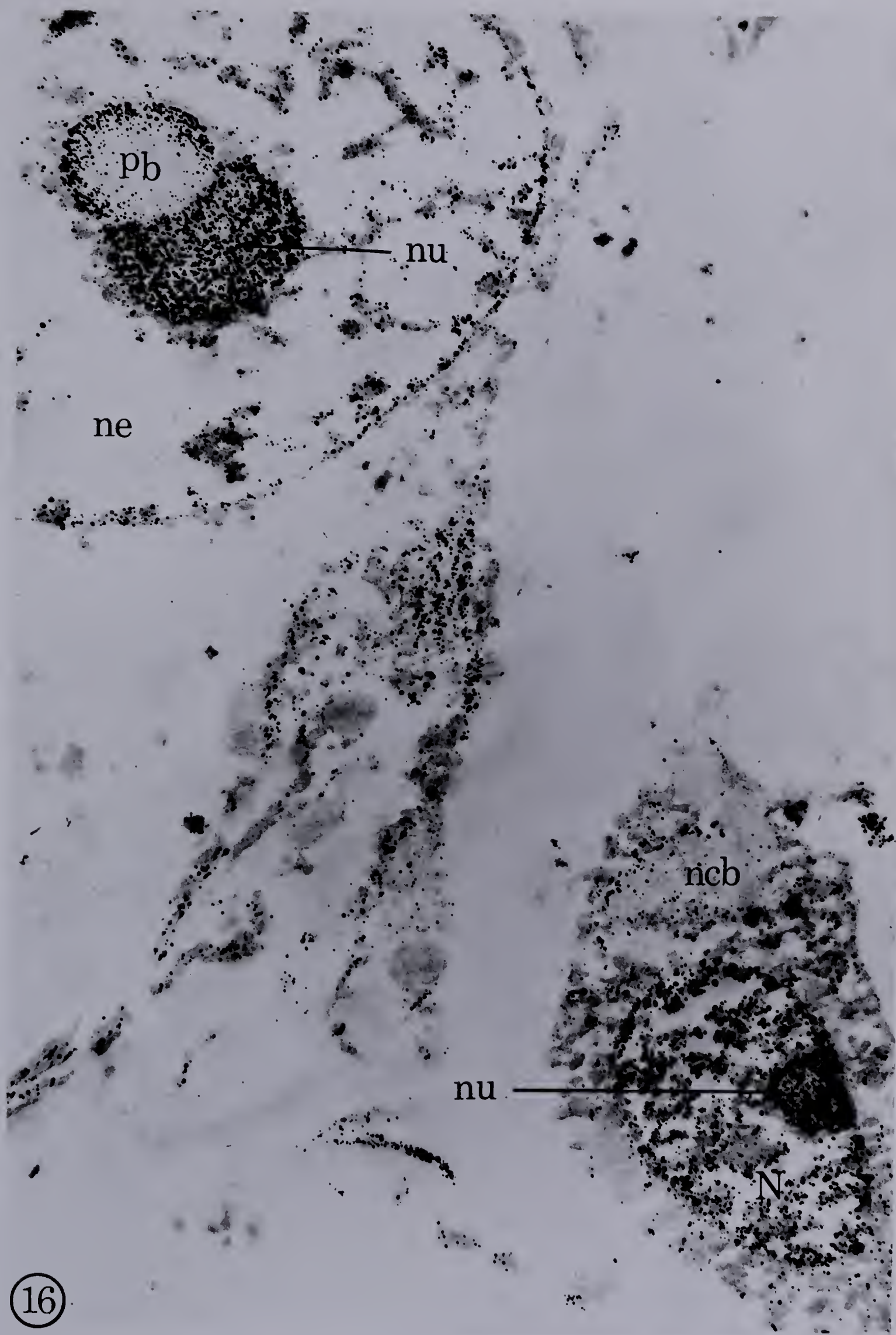




Fig. 17. A cnidocyte showing an undischarged nematocyst (nt)
and the nucleus (Nc).

Picroformol fixation, silver stain and Araldite
embedding, counterstain uranyl acetate.

X 20,000.

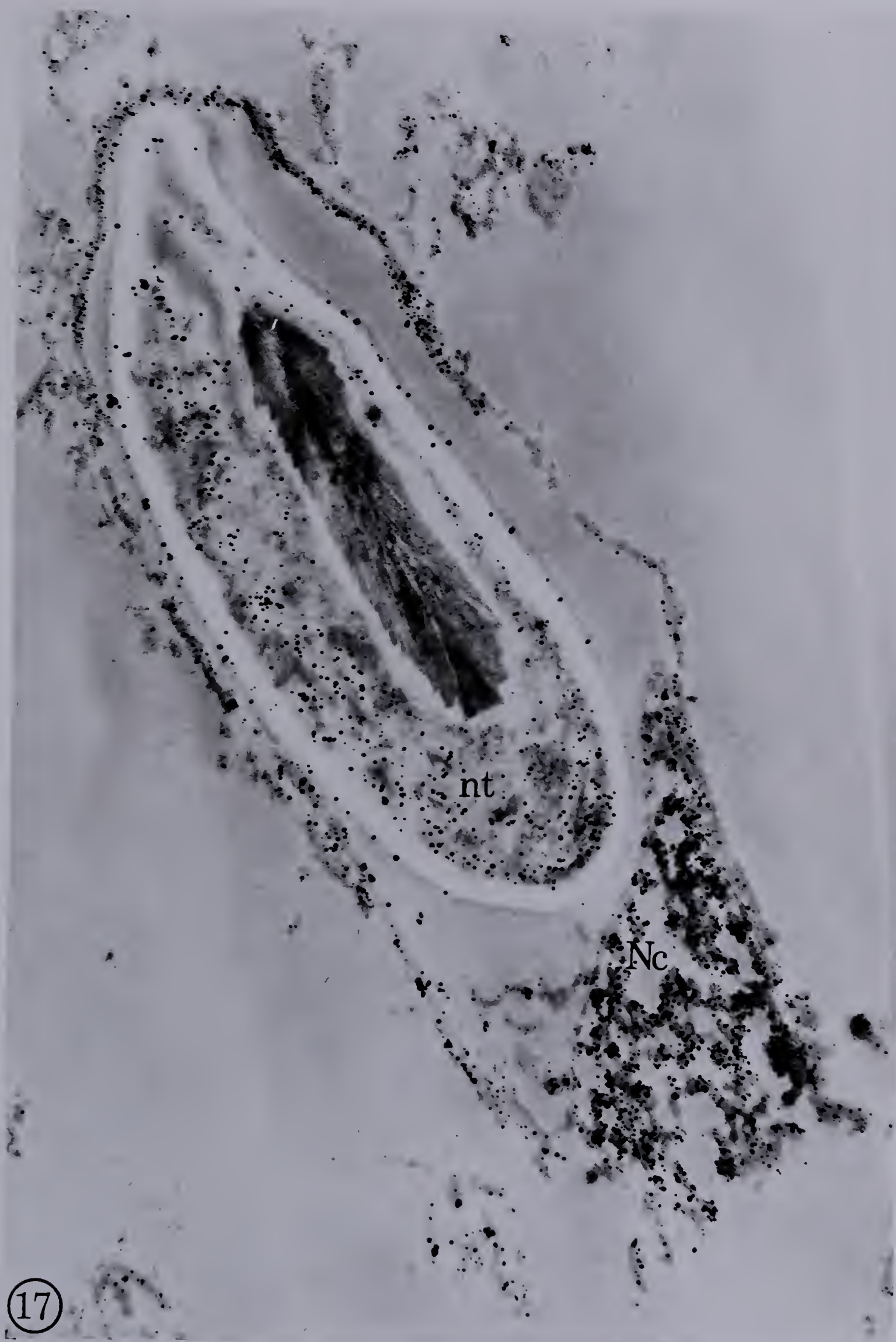


Fig. 18. A nerve cell in the hydranth wall showing a process (p) carrying a sensory hair at its tip, which is projecting externally.

ME, mesoglea; OS, outer surface; p, process; P, pit; sh, sensory hair.

Picroformol fixation, silver stain and Araldite embedding, counterstain uranyl acetate. X 22,000.

Fig. 19. A section through a cnidocil for the comparison with the sensory hair. Note the presence of supporting structures surrounding the cnidocil which are lacking in case of sensory hair (Fig. 18).

cc, cnidocil; OS, outer surface; S, supporting structures.

Picroformol fixation, silver stain and Araldite embedding, counterstaining with uranyl acetate. X 40,000.

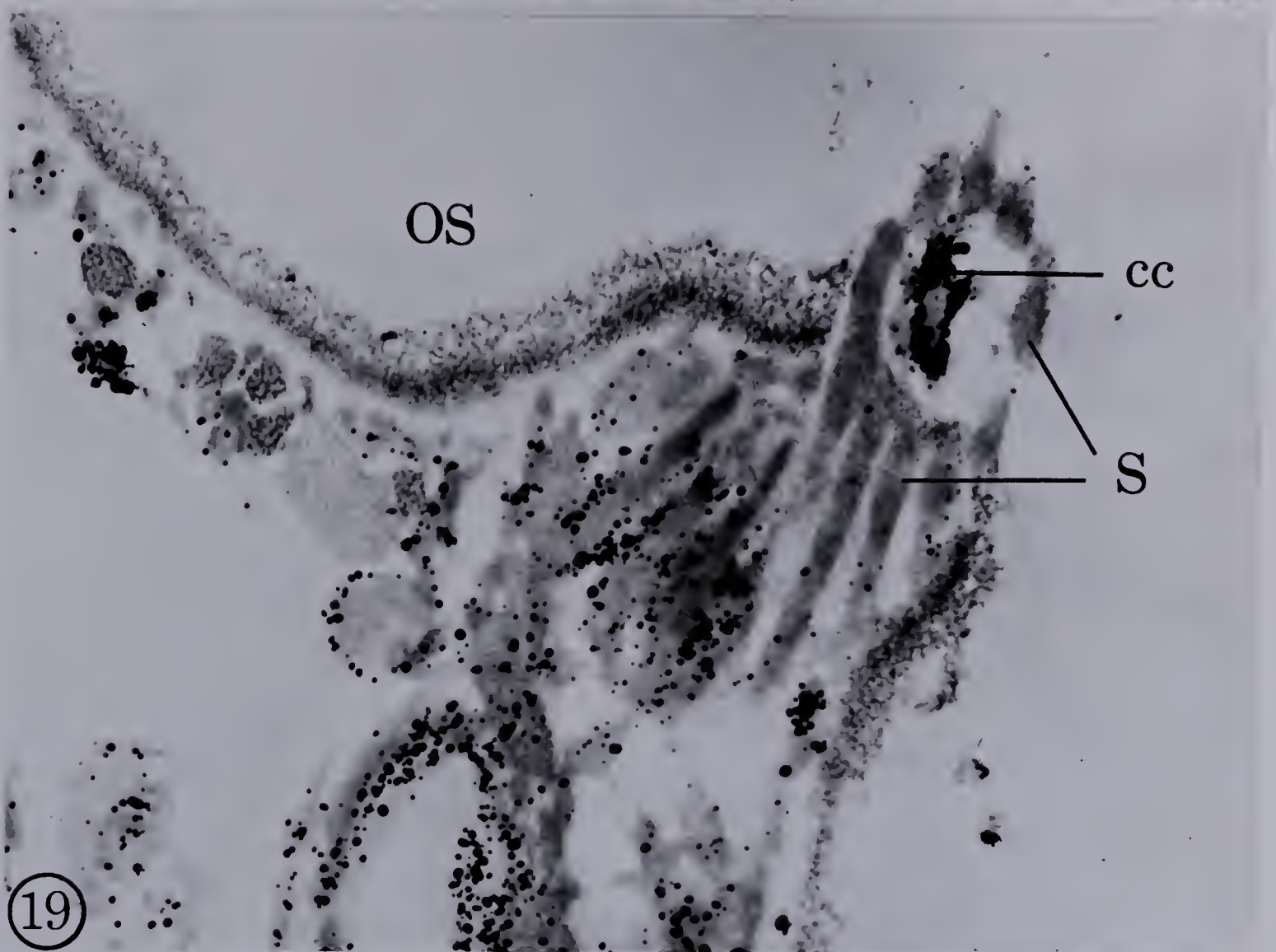
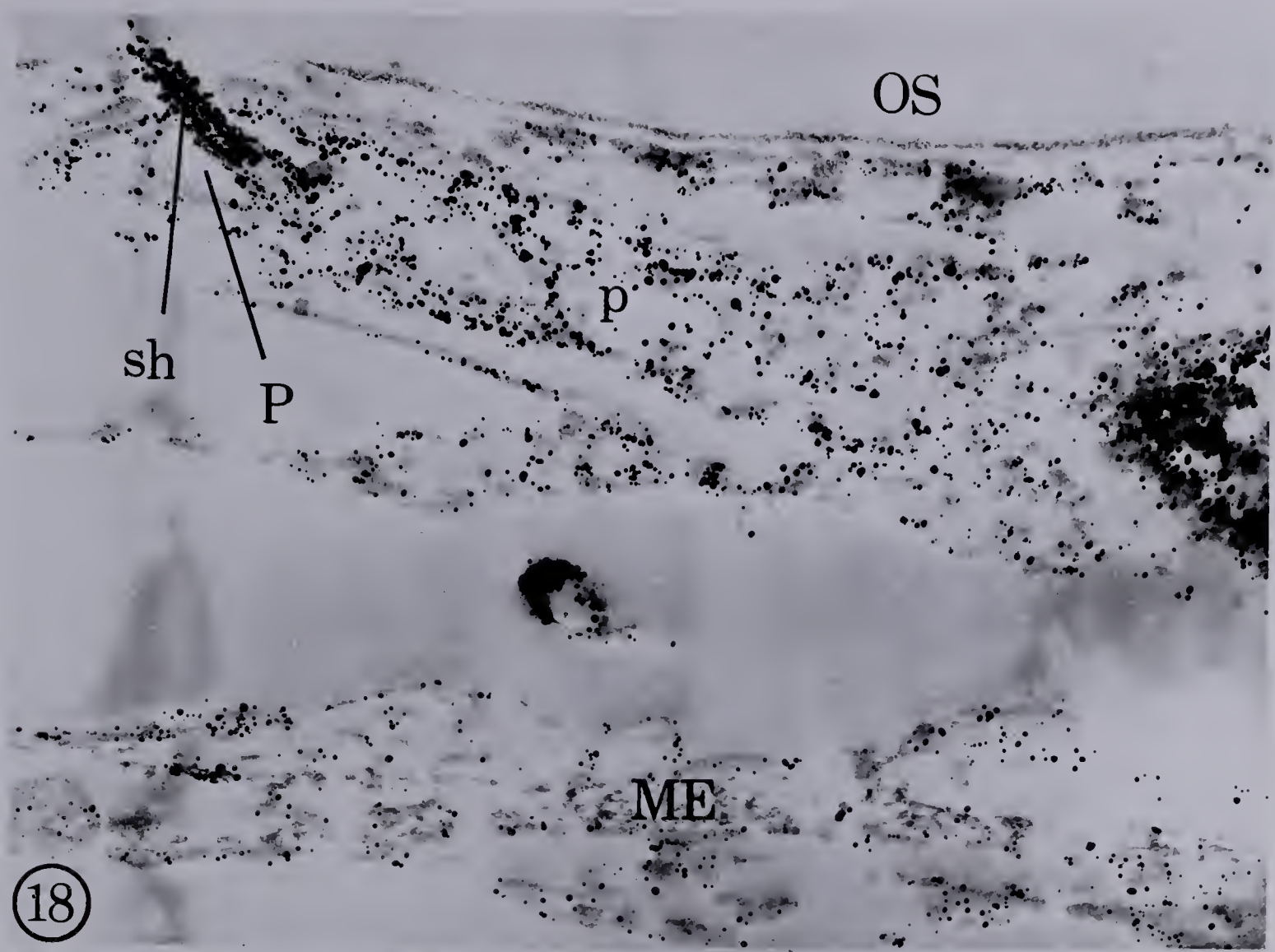


Fig. 20. A section through mesoglea and several muscle processes. X 20,000.

EC, ectoderm; ME, mesoglea; mp, muscle processes.

Fig. 21. An oblique section through the muscle (mp) showing a regular pattern of silver deposition. X 27,000.

Picroformol fixation, silver stain and Araldite embedding, counterstain uranyl acetate.

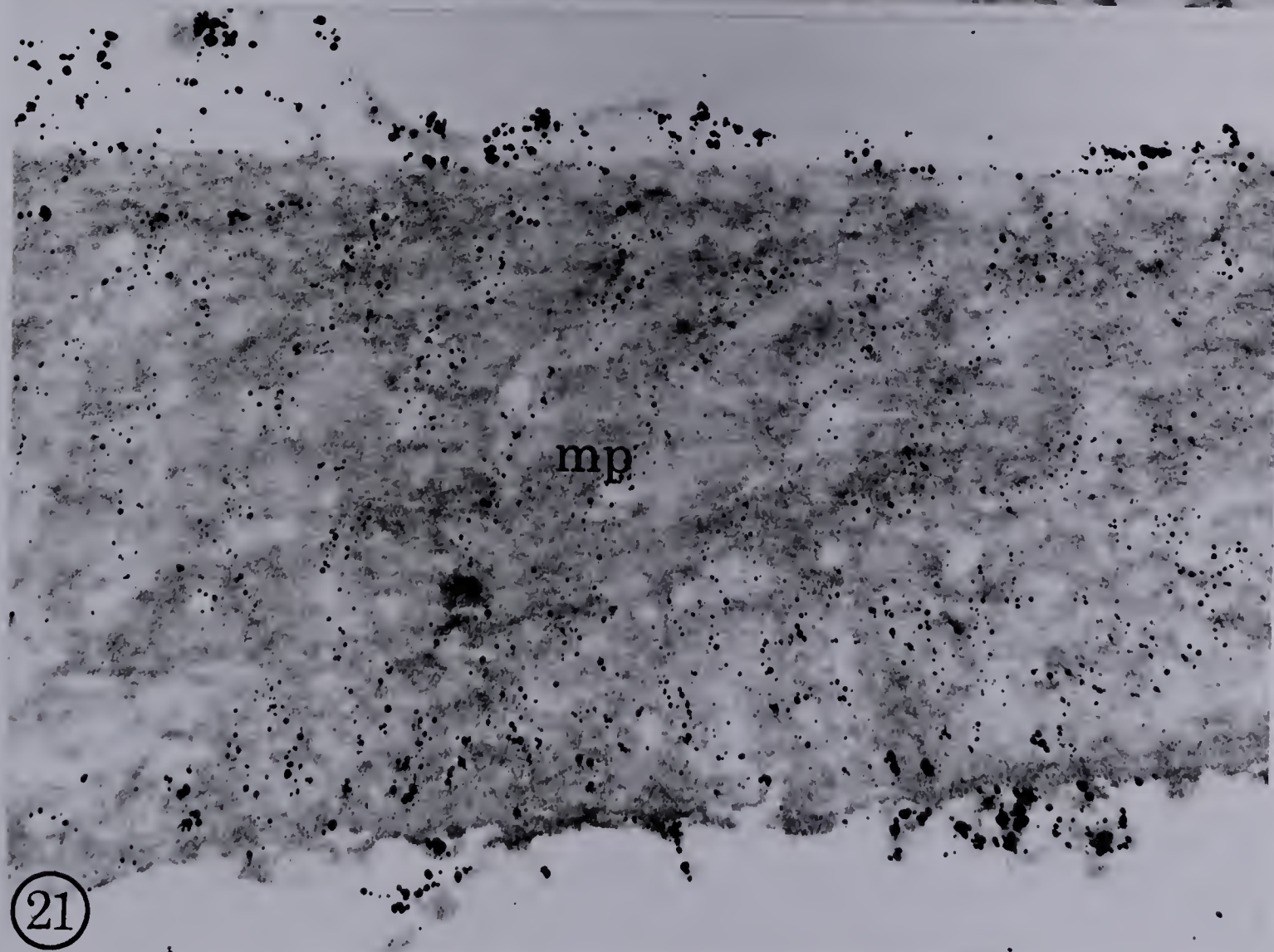
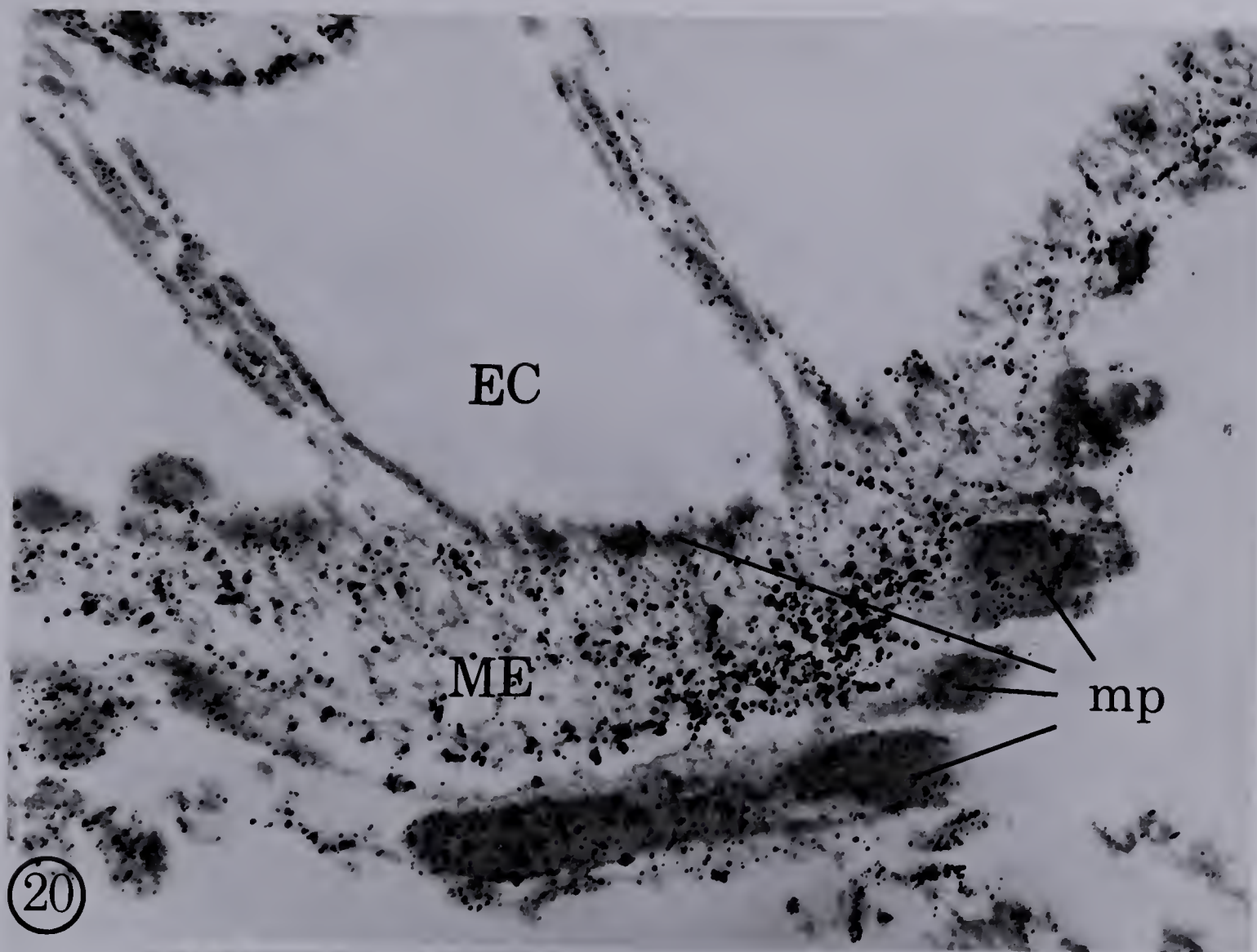


Fig. 22. A diagrammatic representation showing nerve cells and their processes in relation to other structures in the ectoderm.

a, A-type vesicles; bl, large B-type vesicles; bs, small B-type vesicles; c, cnidocyte; cc, cnidocil; cv, C-type vesicles; EC, ectoderm; Er, endoplasmic reticulum; L, lipid body; ME, mesoglea; mp, muscle process; Mv, multivesicular body; N, nerve cell nucleus; n, nerve process; Nc, cnidocyte nucleus; ncb, nerve cell body; ne, ectodermal nucleus; nt, nematocyst; OS, outer surface; P, pit; pb, protein body; r, rootlet; S, supporting structures; sh, sensory hair, ssh, subepithelial sensory hair; V, vacuole in the epitheliomuscular cell.

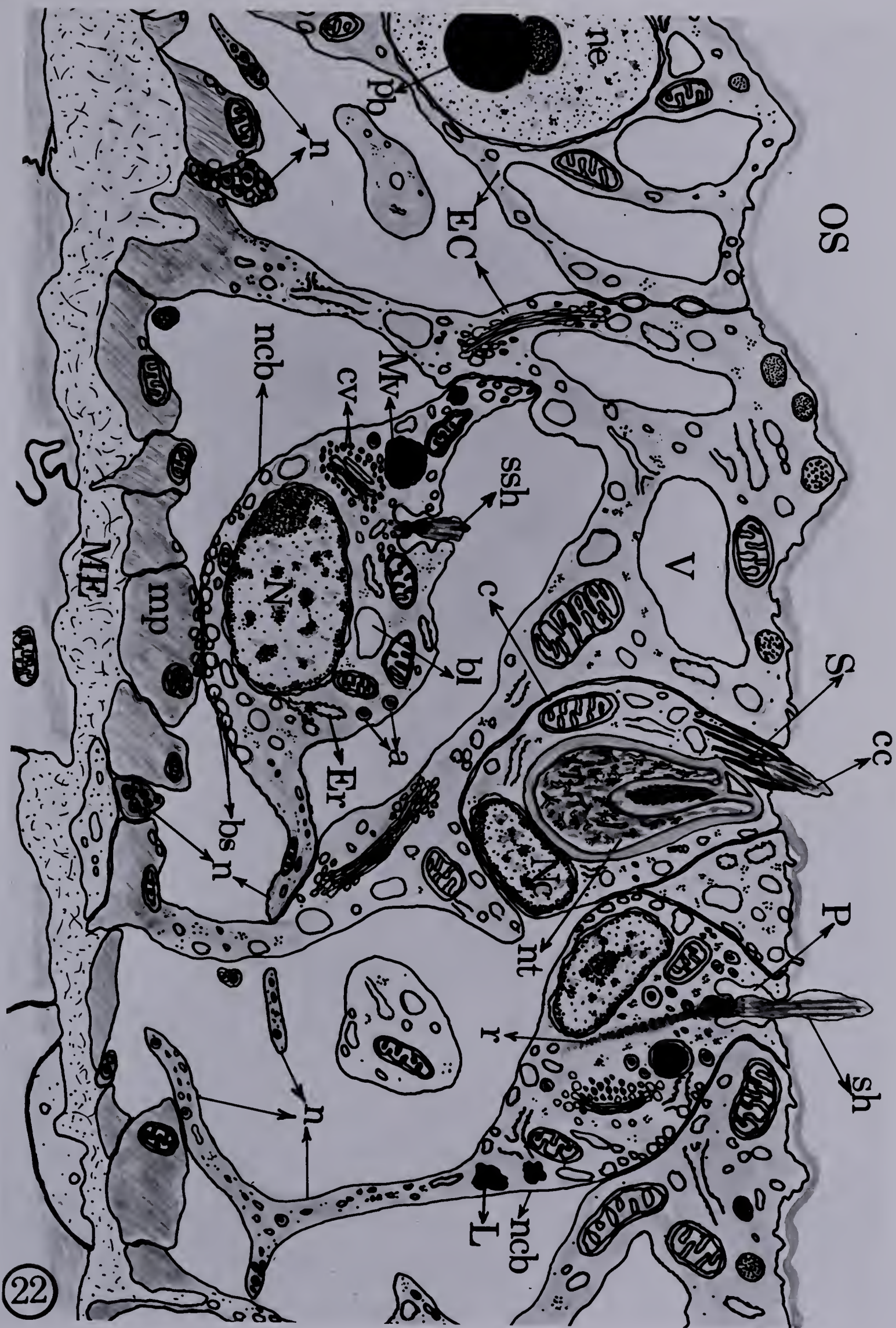


Fig. 22A. A phase contrast micrograph of a typical section of the hydranth wall showing two cellular layers, ectoderm and endoderm separated by mesoglea.

Compare with Fig. 23.

EC, ectoderm; EN, endoderm; ME, mesoglea.

Osmium tetroxide fixation, Araldite embedding and toluidine blue staining. X 3,200.

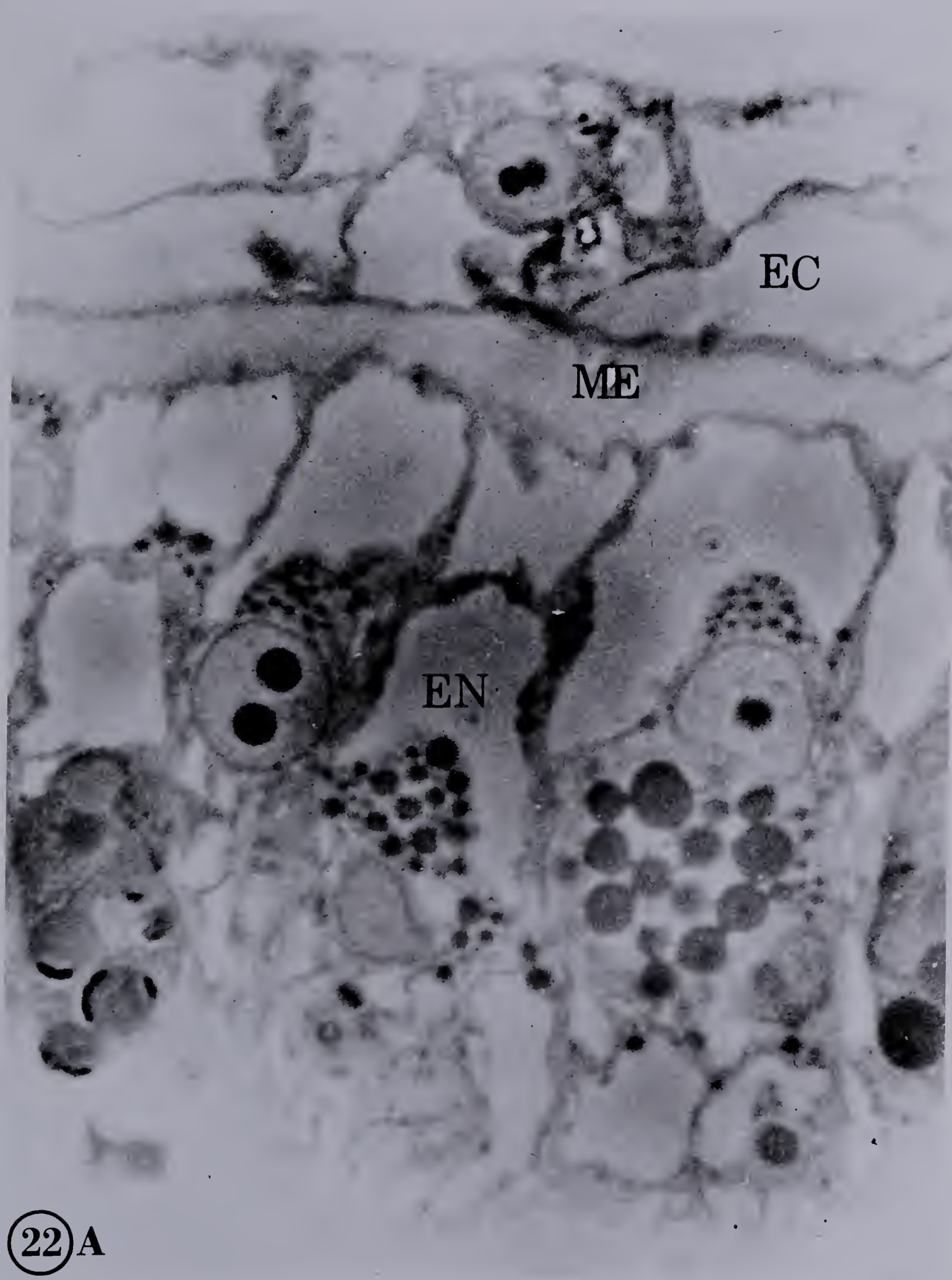


Fig. 23. An electron micrograph of a section similar to Fig. 22A.

EC, ectoderm; EN, endoderm; GC, gland cell; ME, mesoglea; ne, ectodermal nucleus; OS, outer surface.

Osmium tetroxide fixation, Araldite embedding, and uranyl acetate staining. X 10,000.

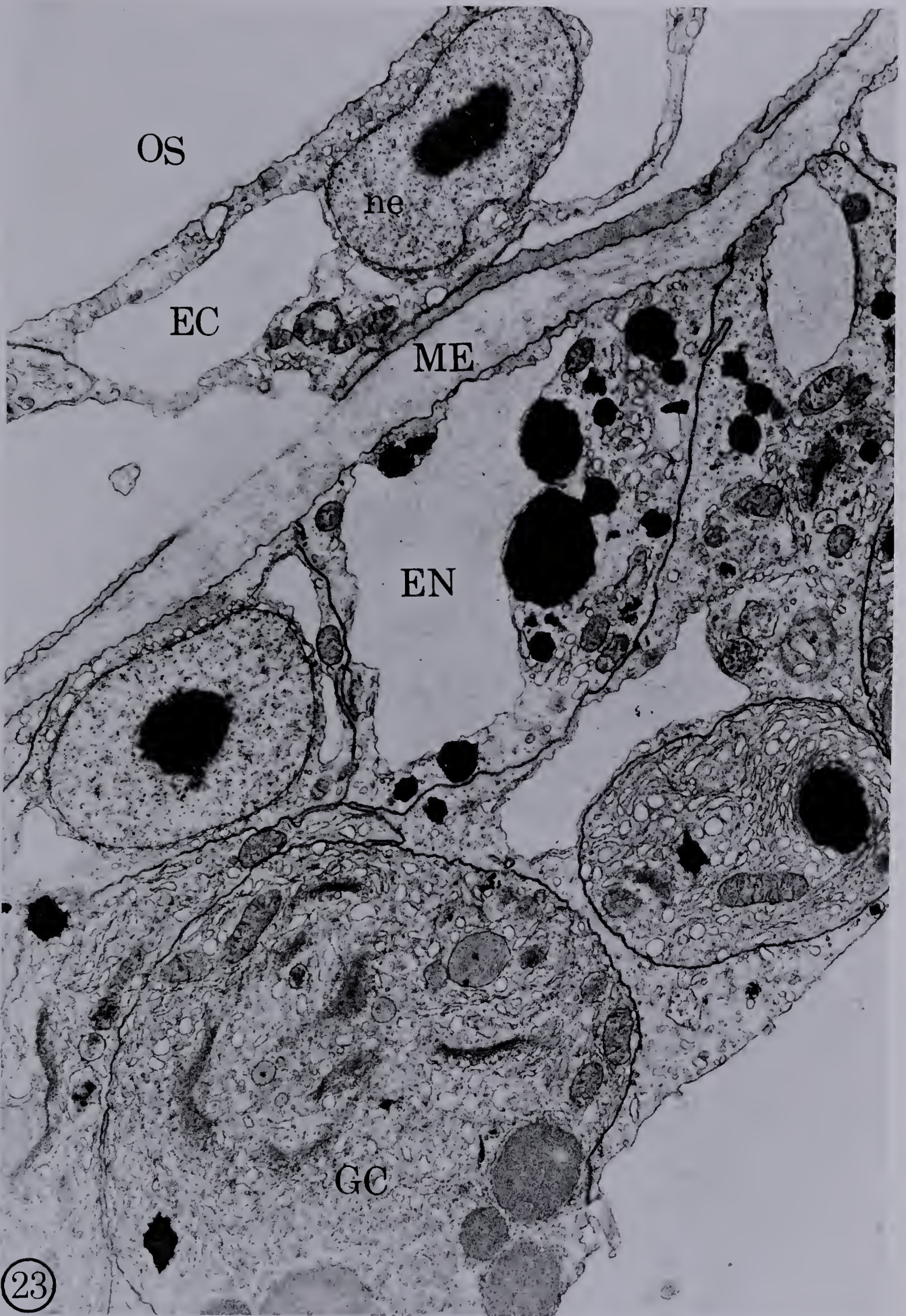
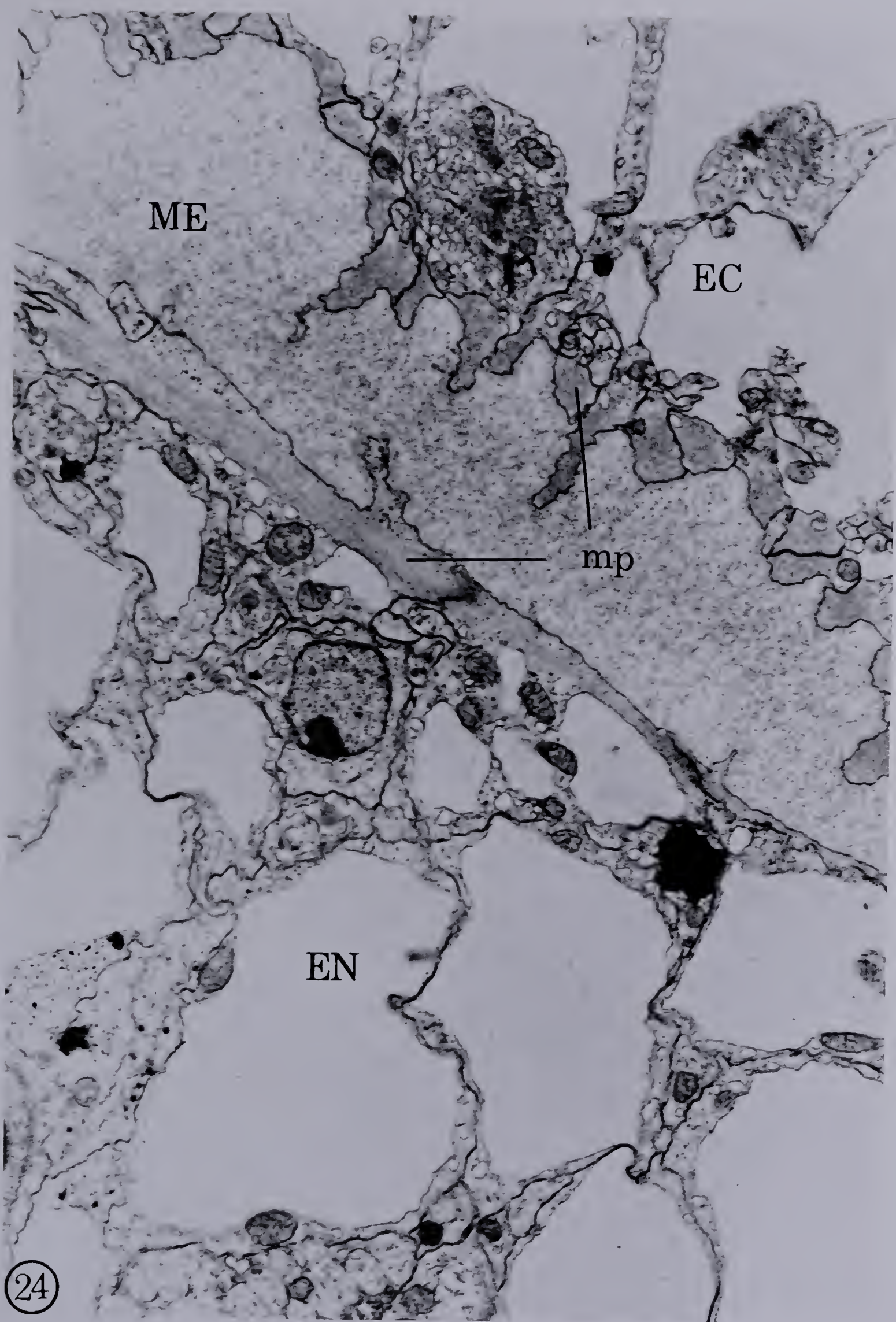


Fig. 24. A cross section through the hypostome region showing the orientation, regular arrangement, location and structure of the muscle processes.

EC, ectoderm; EN, endoderm; ME, mesoglea; mp, muscle processes.

Osmium tetroxide fixation, Araldite embedding and uranyl acetate staining. X 12,000.



Figs. 25 and 26. Electron micrographs of two nerve cells in the hydranth wall, one silver stained (Fig. 25), the other in an osmium fixed preparation (Fig. 26), both showing subepithelial sensory hairs. Note the resemblance in shape and size of the two.

a, A-type vesicles; EC, ectoderm; ncb, nerve cell body; N, nerve cell nucleus; n, nerve process; nu, nucleolus; ME, mesoglea; mp, muscle process; OS, outer surface; P, pit; ssh, subepithelial sensory hair.

Fig. 25. Picroformol fixation, silver stain and Araldite embedding. X 22,000.

Fig. 26. Osmium tetroxide fixation, Araldite embedding and uranyl acetate staining. X 22,500.

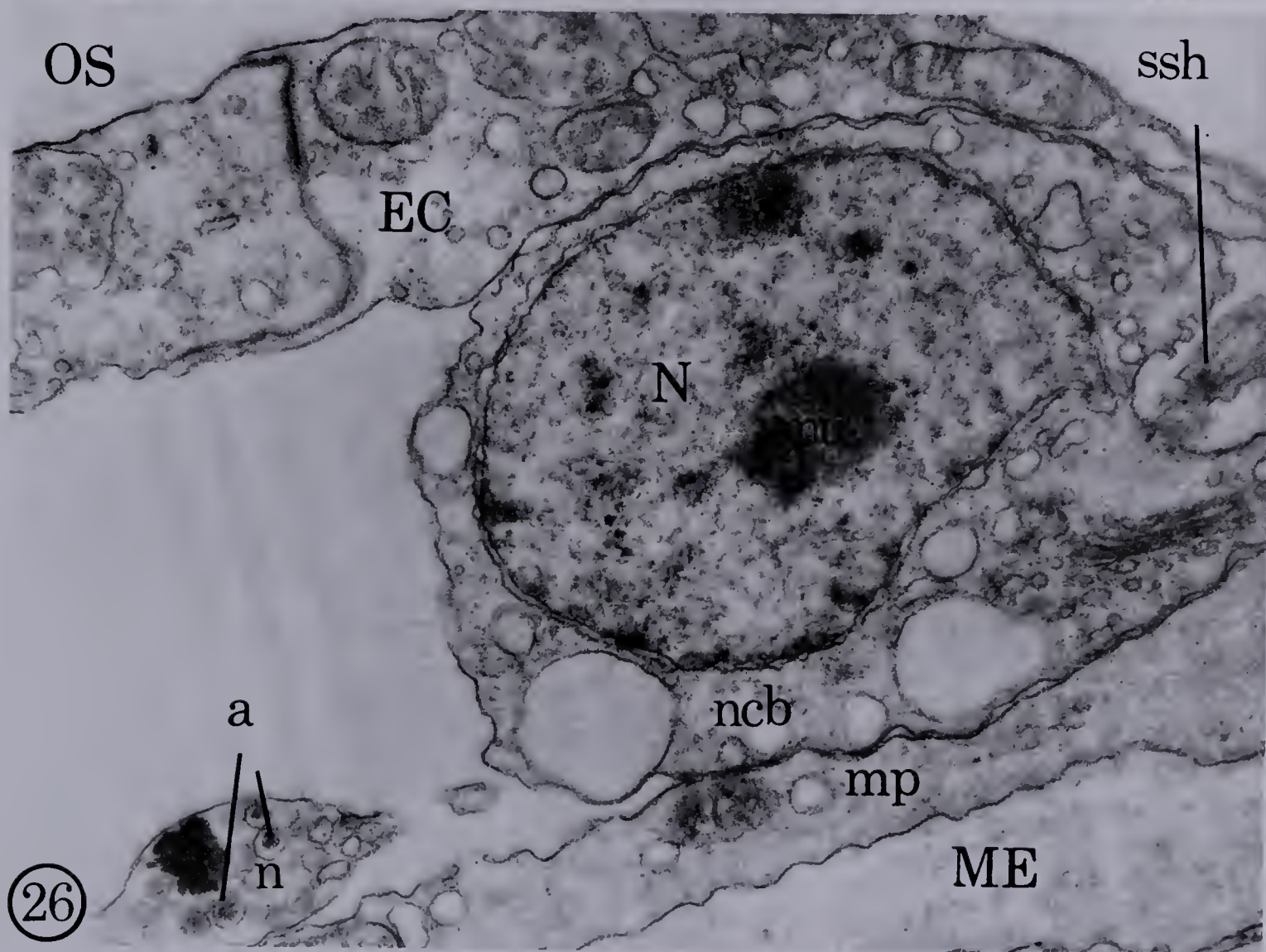
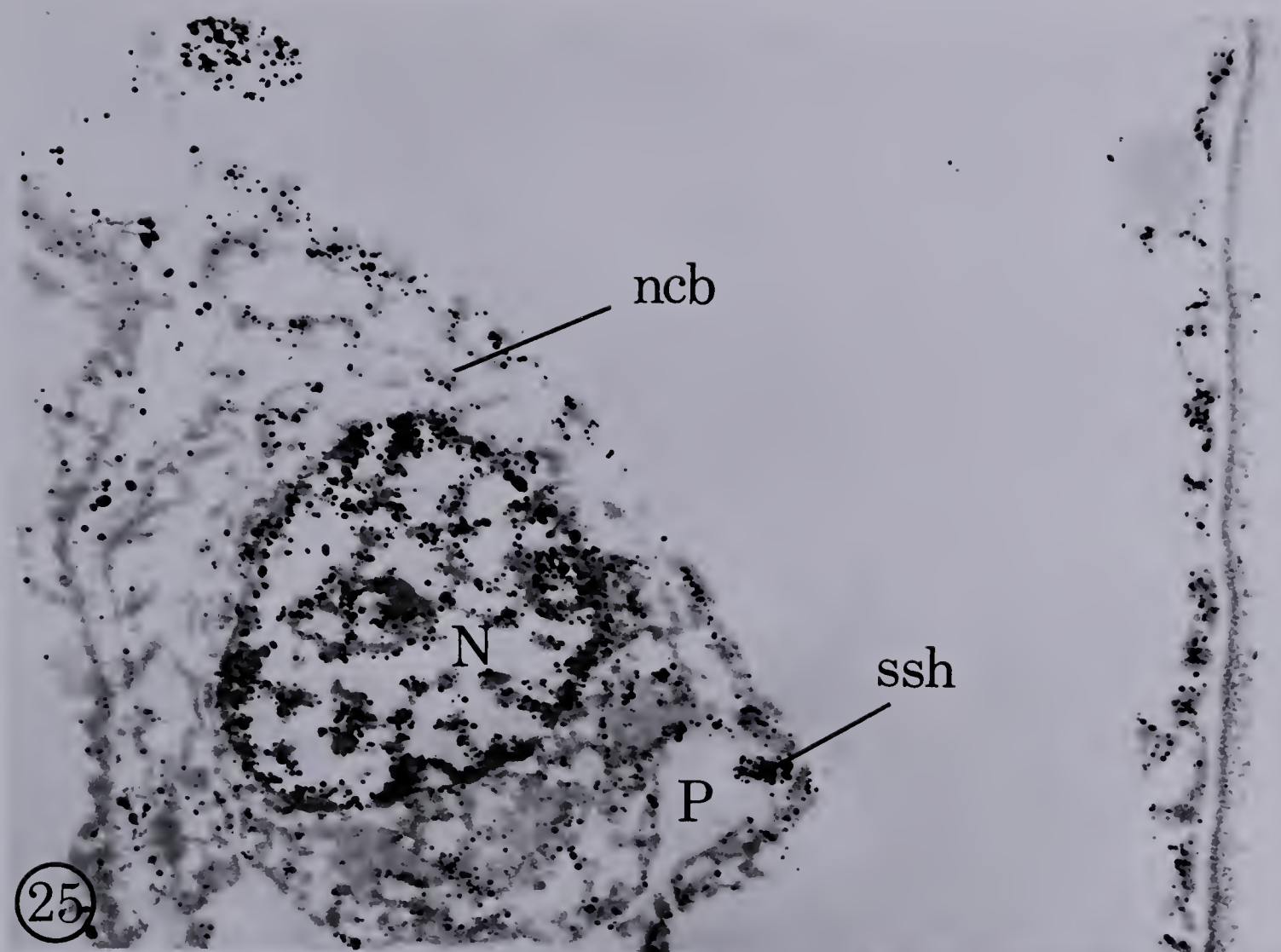


Fig. 27. A section of the tentacle showing a nerve cell with a subepithelial sensory hair and an ectodermal nucleus for comparison.

bs, small B-type vesicles; EC, ectoderm; mp, muscle process; ne, ectodermal nucleus; N, nerve cell nucleus; ncb, nerve cell body; ssh, subepithelial sensory hair; V, vacuole.

Osmium tetroxide fixation, Araldite embedding and uranyl acetate staining. X 16,500.

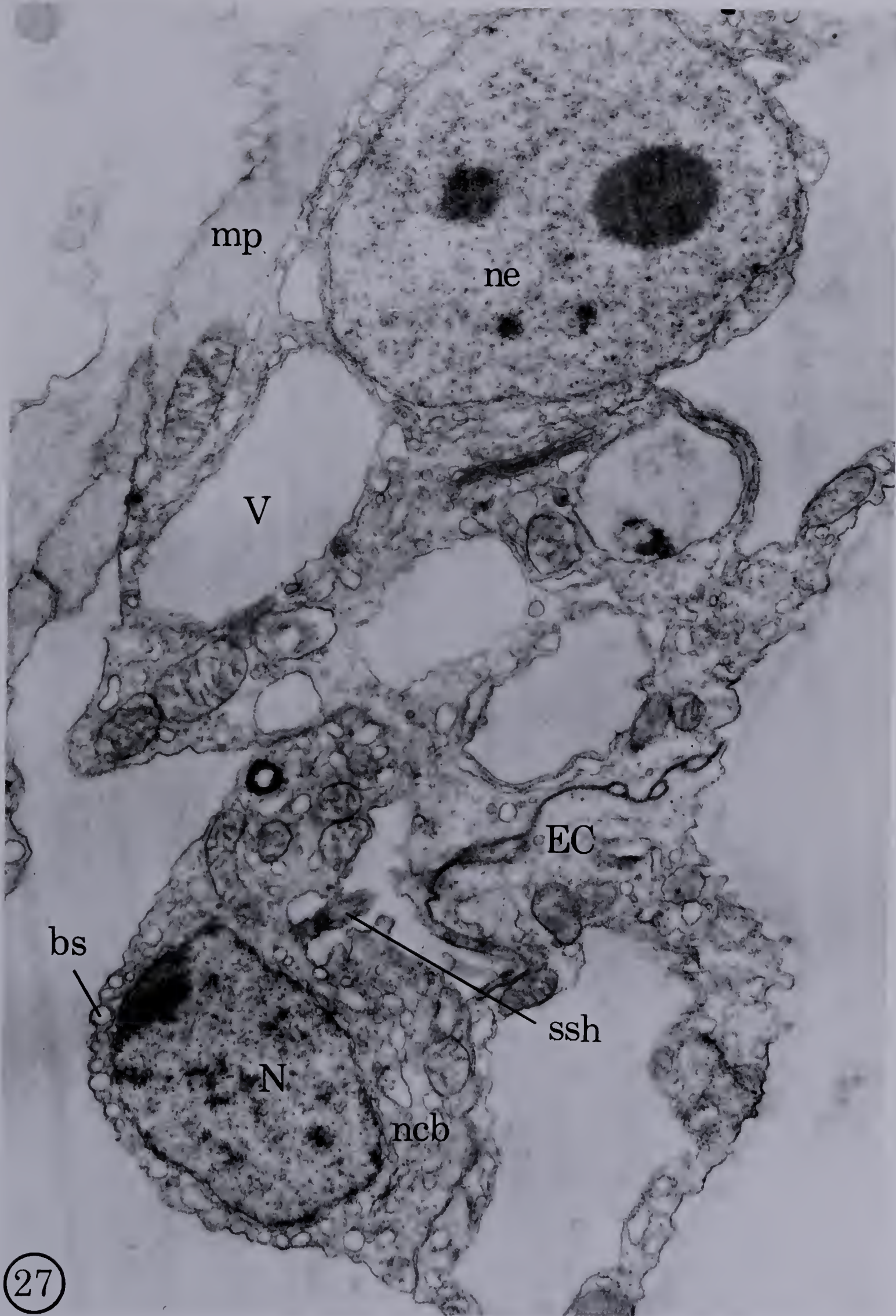


Fig. 28. A nerve cell in the ectoderm showing a subepithelial sensory hair in cross section, and a section of the rootlet.

EC, ectoderm; M, mitochondrion; N, nerve cell nucleus; P, pit; r, rootlet; ssh, subepithelial sensory hair.

Osmium tetroxide fixation (veronal acetate buffer, containing sucrose), Araldite embedding, uranyl acetate staining. X 25,000.

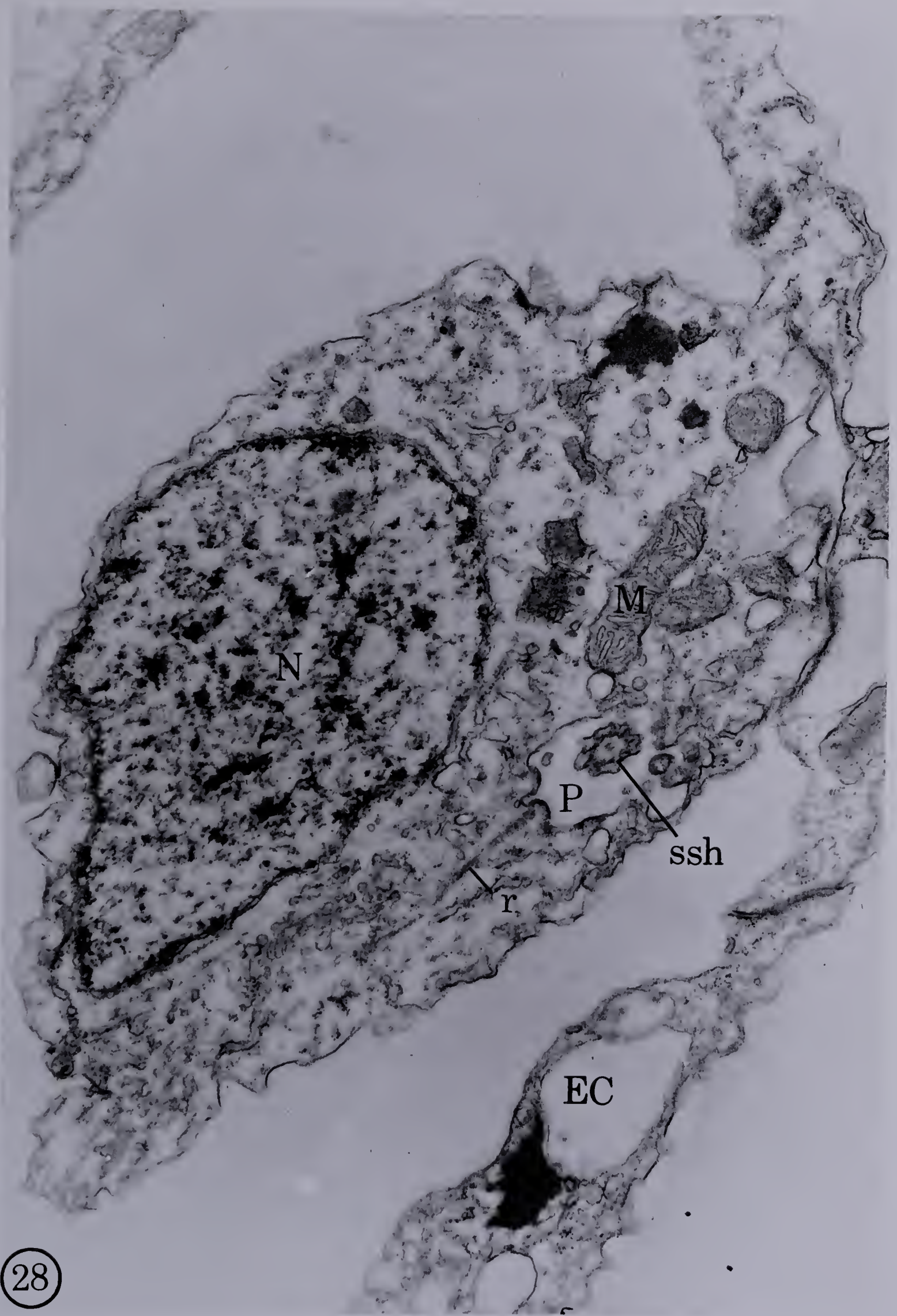


Fig. 29. A section through the tentacle showing a group of nerve cells with a cnidocyte among them. Note that the cell membranes of the cnidocyte and one of the nerve cells are in close contact and are running parallel to each other for a considerable distance.

a, A-type vesicles; bs, small B-type vesicles;
c, cnidocyte; EC, ectoderm; mp, muscle process;
N, nerve cell nucleus; n, nerve process; ncb, nerve cell body; Nc, cnidocyte nucleus; nu, nucleolus;
OS, outer surface; P, pit; r, rootlet; S, supporting structures; sh, sensory hair.

Osmium tetroxide fixation, Araldite embedding, and uranyl acetate staining. X 16,000.

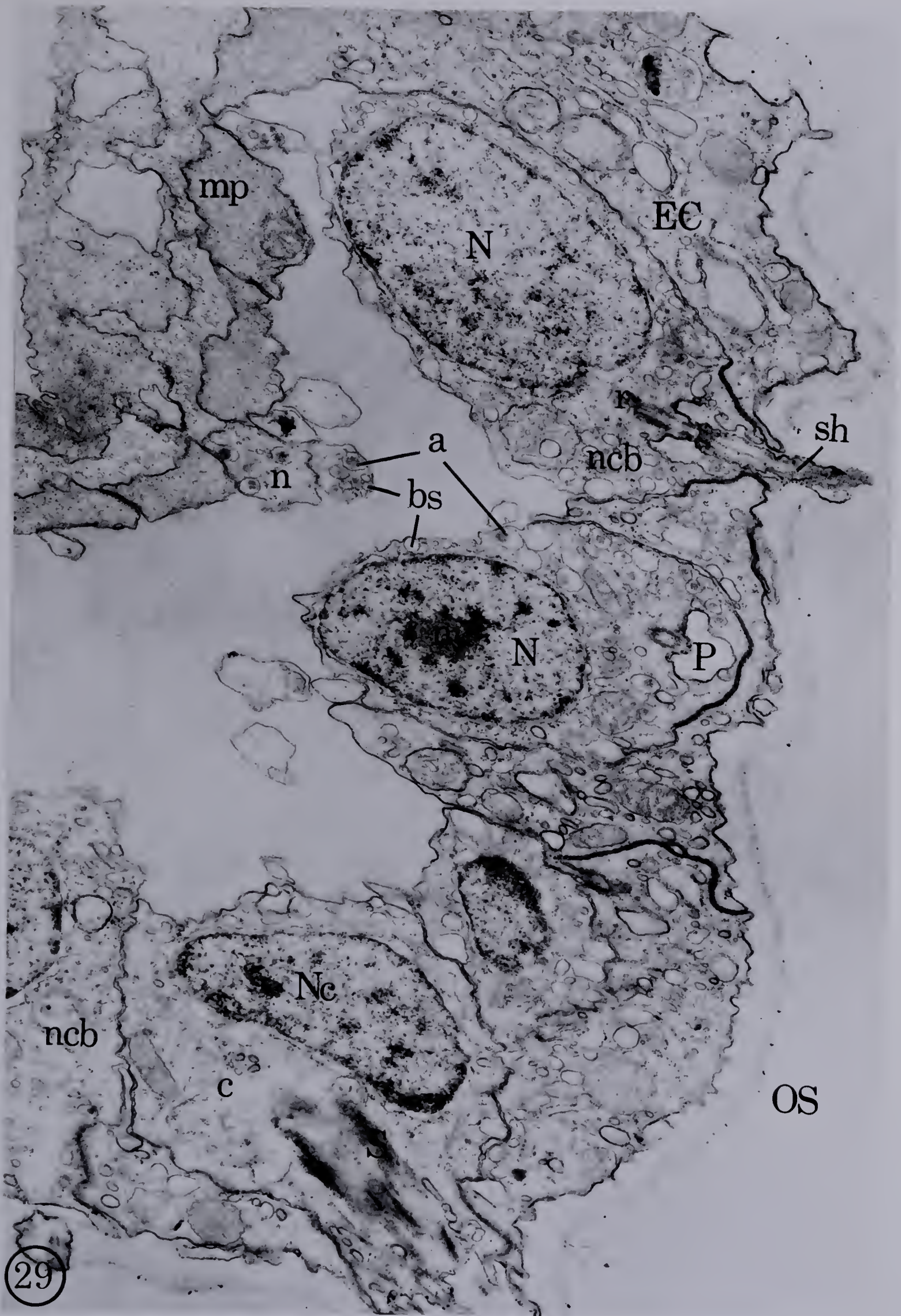




Fig. 30. A section of a nerve cell with a sensory hair projecting externally, showing a large B-type vesicle.

bl, large B-type vesicle; bs, small B-type vesicles; EC, ectoderm; Er, endoplasmic reticulum; N, nerve cell nucleus; V, vacuole in the ectodermal epitheliomuscular cell.

Osmium tetroxide fixation, Araldite embedding and uranyl acetate staining. X 15,000.

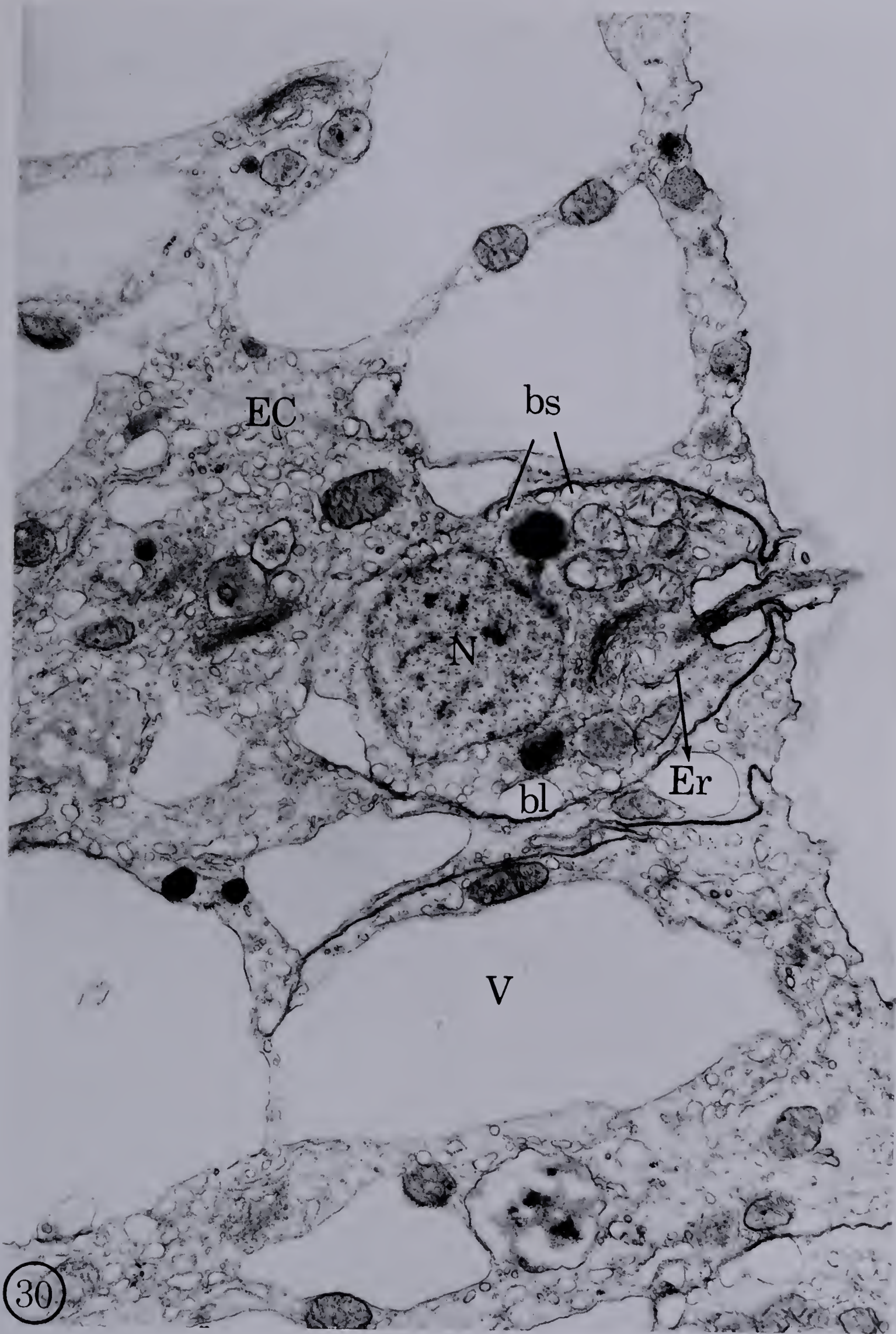




Fig. 31. A section of a nerve cell showing a multivesicular body of unknown nature, in the Golgi area.

bl, large B-type vesicle; Mv, multivesicular body;
G, vesicles of Golgi complex; ncb, nerve cell body;
V, vacuole in the epithelio-muscular cell.

Osmium tetroxide fixation; Araldite embedding.

X 16,200.

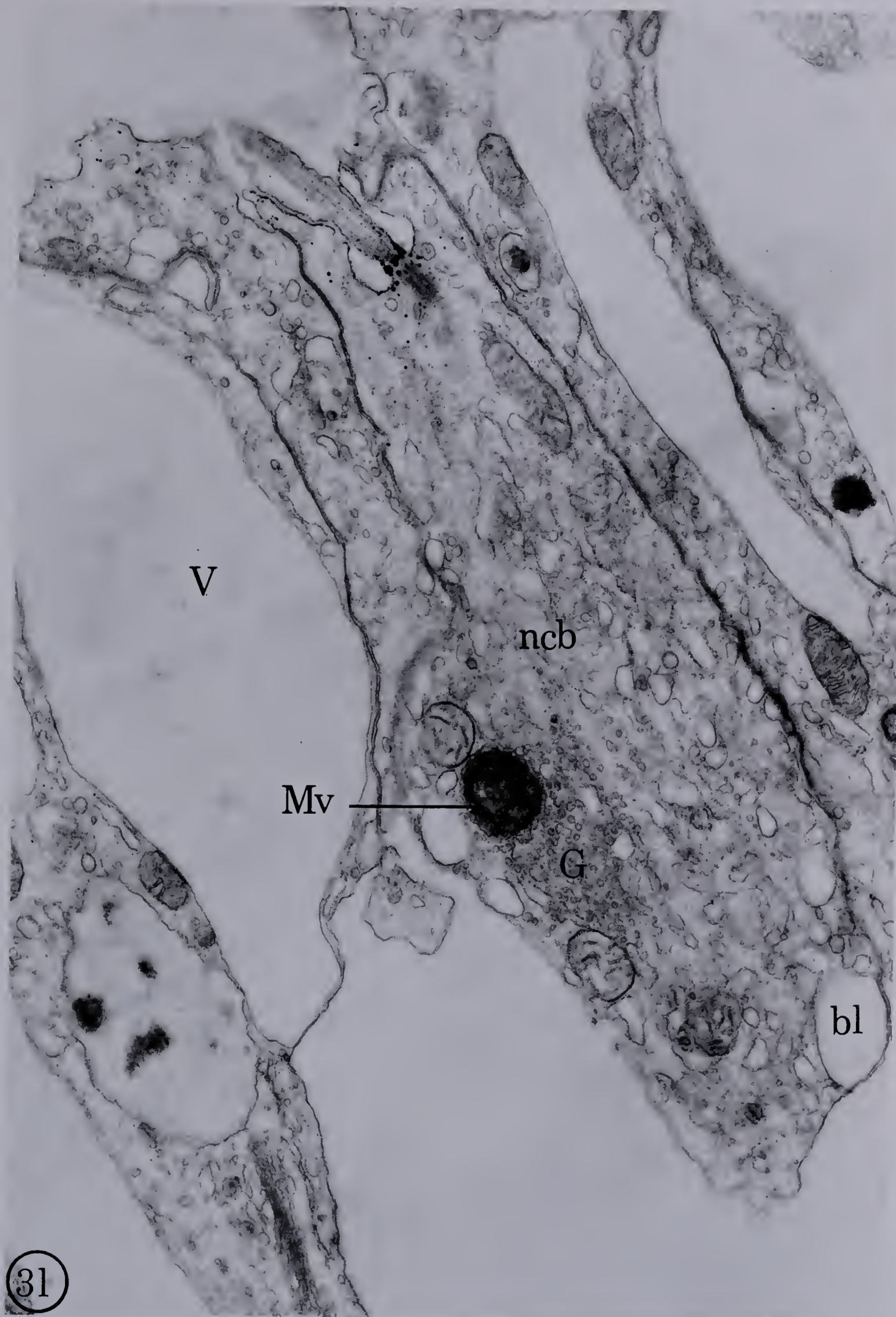


Fig. 32. A nerve cell showing a sensory hair projecting externally, centrioles and the banded rootlet. Note the septate desmosomal attachments with the epithelio-muscular cells on both sides.

EC, ectoderm, C, centrioles; D, desmosome; M, mitochondrion; N, nucleus; P, pit; r, rootlet; sh, sensory hair.

Osmium tetroxide fixation, Araldite embedding and uranyl acetate staining. X 36,000.

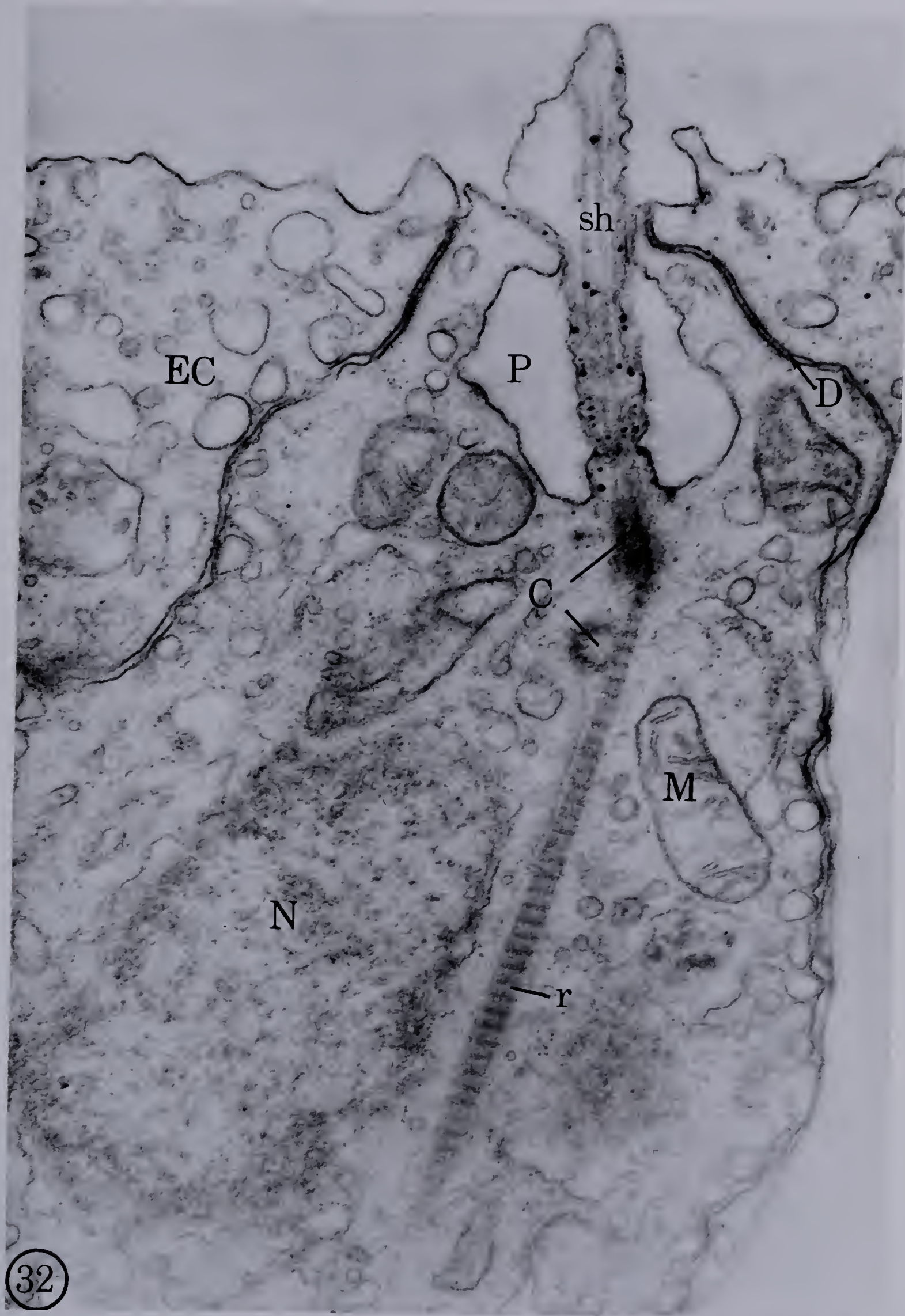
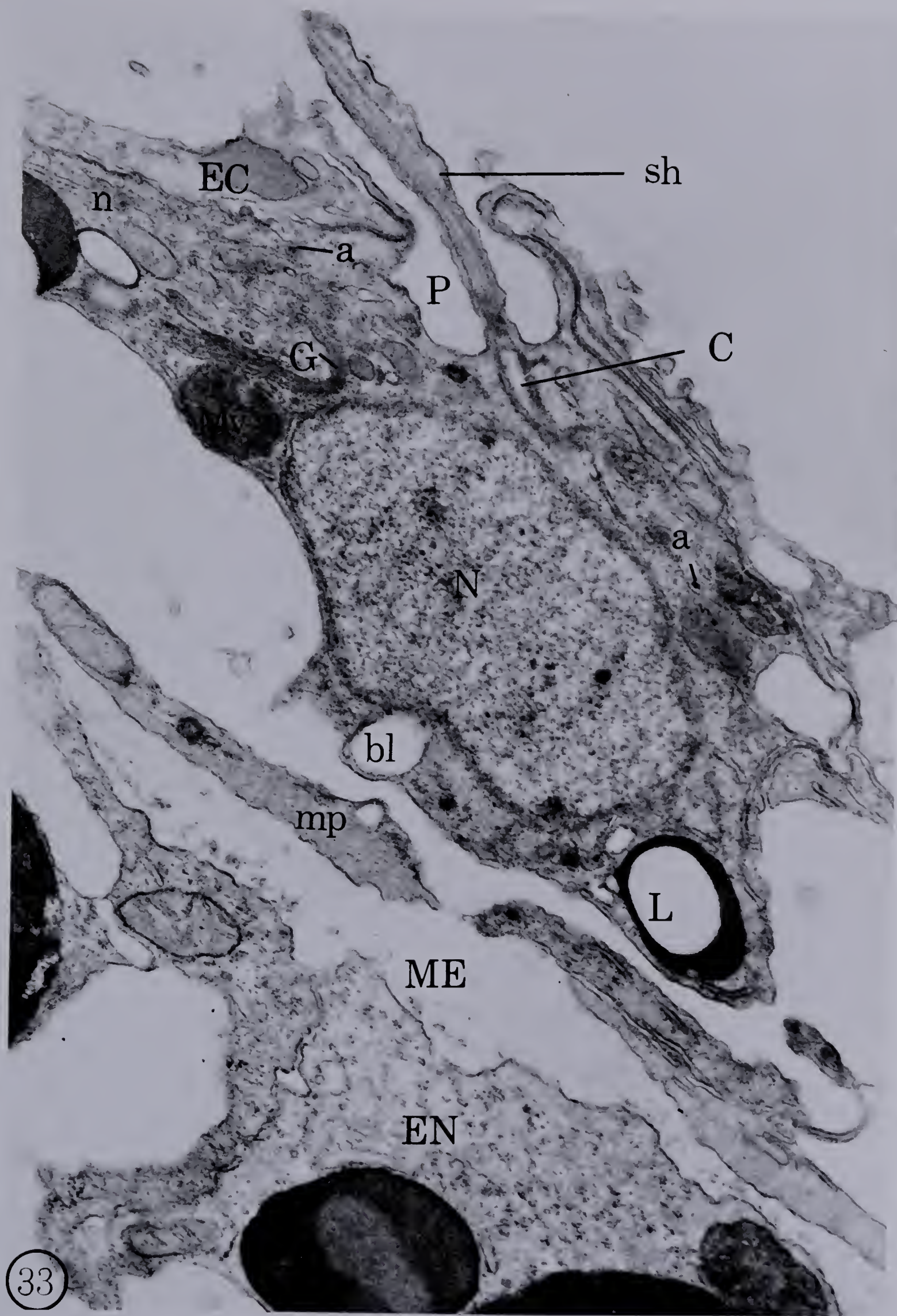


Fig. 33. A nerve cell with a sensory hair and a nerve process after glutaraldehyde-osmium tetroxide fixation.

a, A-type vesicle; bl, large B-type vesicle;
C, centriole; EC, ectoderm; EN, endoderm, G, Golgi
complex; L, lipid body; ME, mesoglea; mp, muscle
process; Mv, multivesicular body; N, nerve cell
nucleus; n, nerve process; P, pit; sh, sensory hair.

Araldite embedding and uranyl acetate staining.

X 24,000.



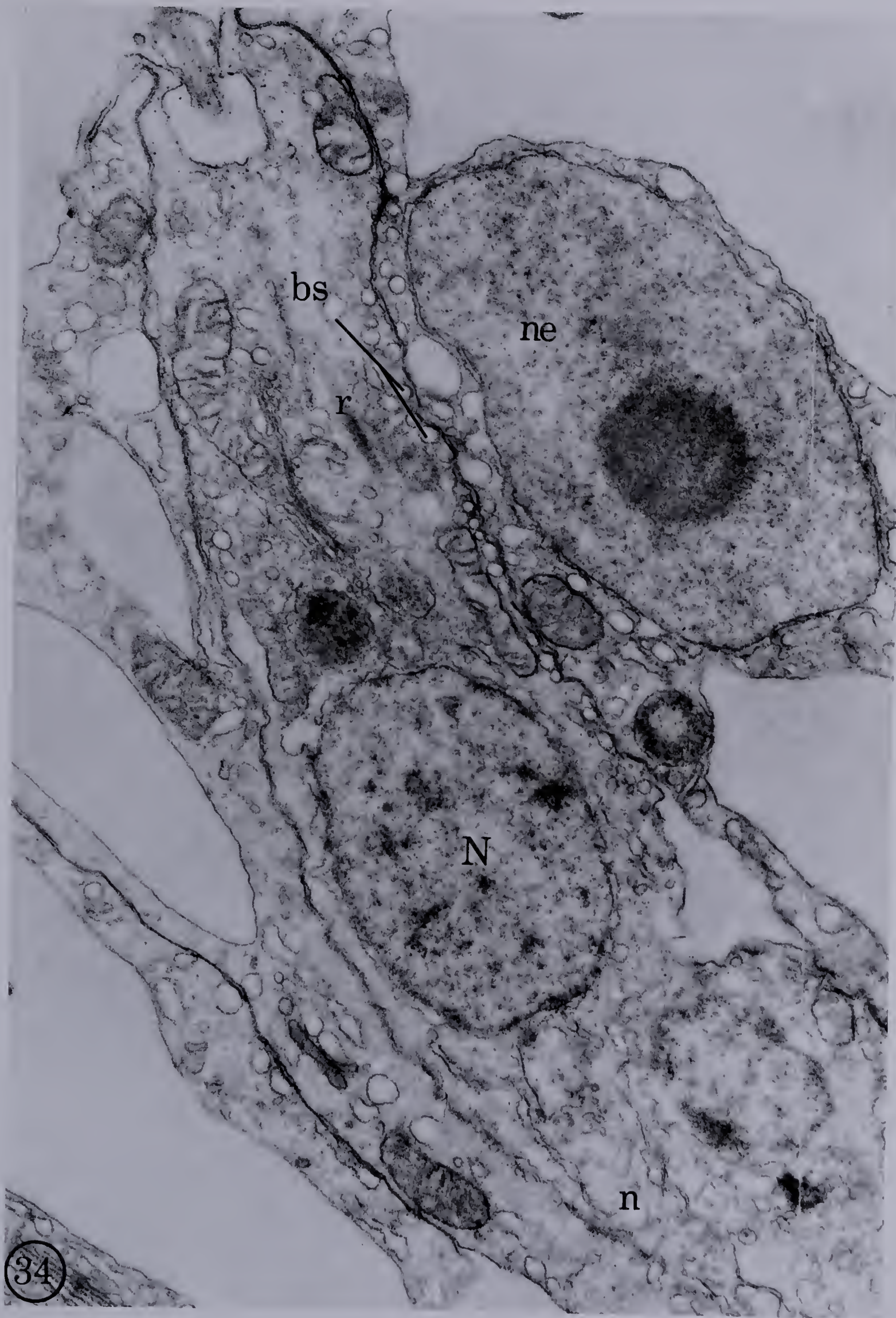


and the same day of the month of June, 1841, the
said John C. Smith, of the County of ... State of ...
do hereby certify that the within and foregoing is a true
and correct copy of the original of the same as the same
now remains in the office of the said John C. Smith.
In testimony whereof, I have hereunto set my hand and
the seal of the said County, at the City of ... this ... day
of June, 1841.

Fig. 34. A nerve cell with a sensory hair at the distal end and a nerve process at the base. Note the small B-type vesicles along the nerve cell membrane in close contact with the cell membrane of an epithelio-muscular cell.

bs, small B-type vesicles; N, nerve cell nucleus;
n, nerve process; ne, ectodermal nucleus; r, rootlet.

Osmium tetroxide fixation, Araldite embedding, and uranyl acetate staining. X 17,000.



Handwritten text, likely a letter or document, written in cursive script. The text is extremely faint and illegible due to the quality of the scan. It appears to be a single page of writing, possibly a letter, with a header section at the top and a main body of text below. There are some markings at the bottom left that could be a signature or initials.

Fig. 35. A section of a nerve cell with a nerve process.

Note the abundance of ribosomes in the nerve cell body.

cv, C-type vesicles; EC, ectoderm; Er, endoplasmic reticulum; L, lipid body; mp, muscle process; N, nerve cell nucleus; n, nerve process; NP, nuclear pore.

Osmium tetroxide fixation, Araldite embedding and uranyl acetate staining. X 17,000.



Handwritten text, likely a letter or document, written in cursive script. The text is faint and mostly illegible due to the quality of the scan. It appears to be a formal document, possibly a letter of introduction or a business correspondence. The text is organized into several paragraphs, with some lines indented. There are some words that are more legible than others, such as "Dear Sir" at the beginning of the first paragraph, and "Yours truly" at the end of the second paragraph. The document is dated "18th Nov 1841" in the bottom right corner.

Fig. 36. A section through a nerve cell body lying in close contact with a muscle process. Note the presence of small B-type vesicles along the nerve cell membrane in close contact with the muscle process and the epithelio-muscular cell. Few vesicles are also seen in the muscle process along the point of contact with the nerve cell body.

bs, small B-type vesicles; cv, C-type vesicles;
EC, ectoderm; ME, mesoglea; mp, muscle process;
N, nerve cell nucleus; OS, outer surface.

Osmium tetroxide fixation, Araldite embedding and uranyl acetate staining. X 19,000.



Fig. 37. A section of an ectodermal nucleus showing its typical structure. Note the presence of a fibrillar structure and a protein body in addition to a nucleolus.

fp, fibrillar structure; ME, mesoglea; ne, ectodermal nucleus; nu, nucleolus; OS, outer surface; pb, protein body.

Osmium tetroxide fixation, Araldite embedding, and uranyl acetate staining. X 28,000.

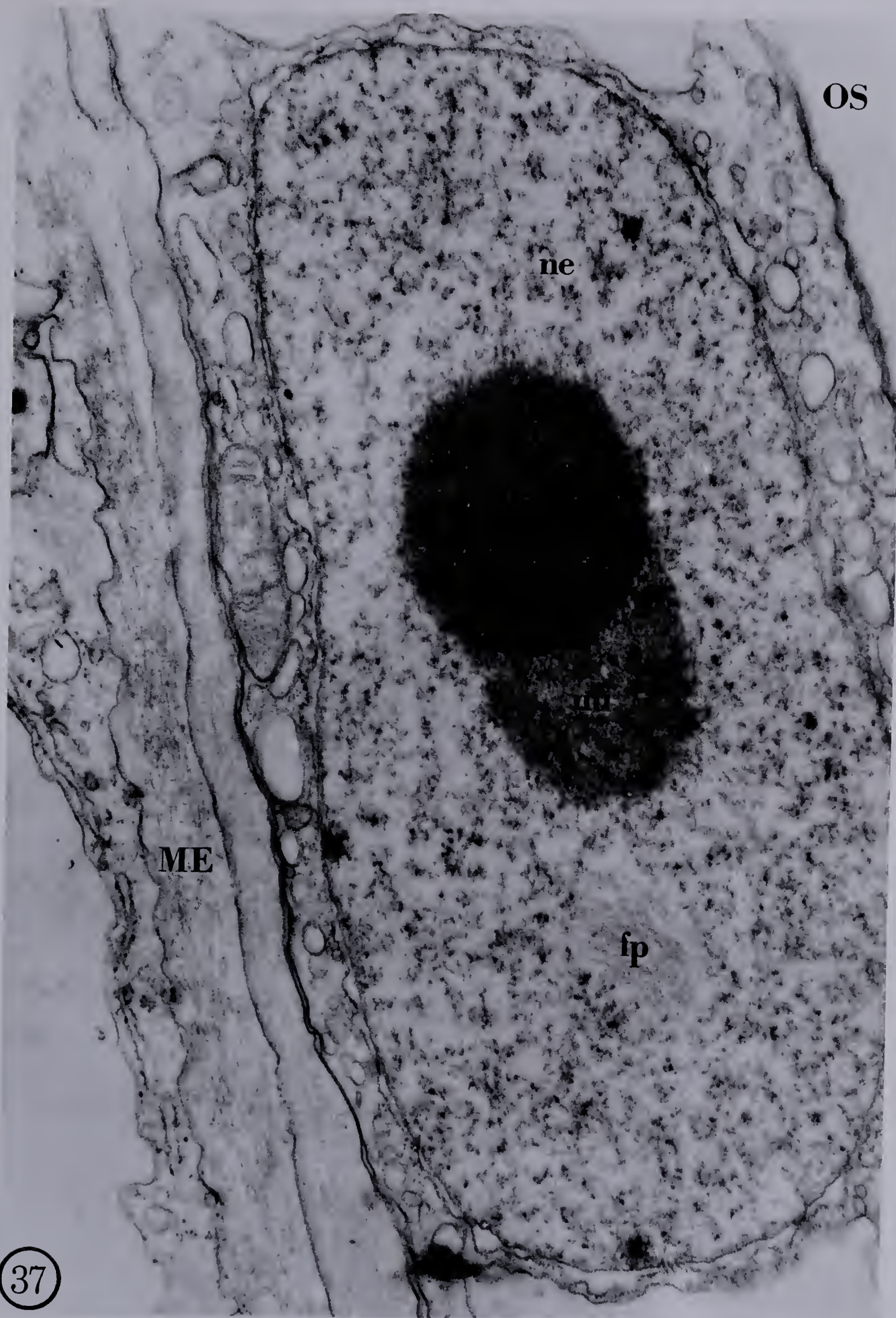
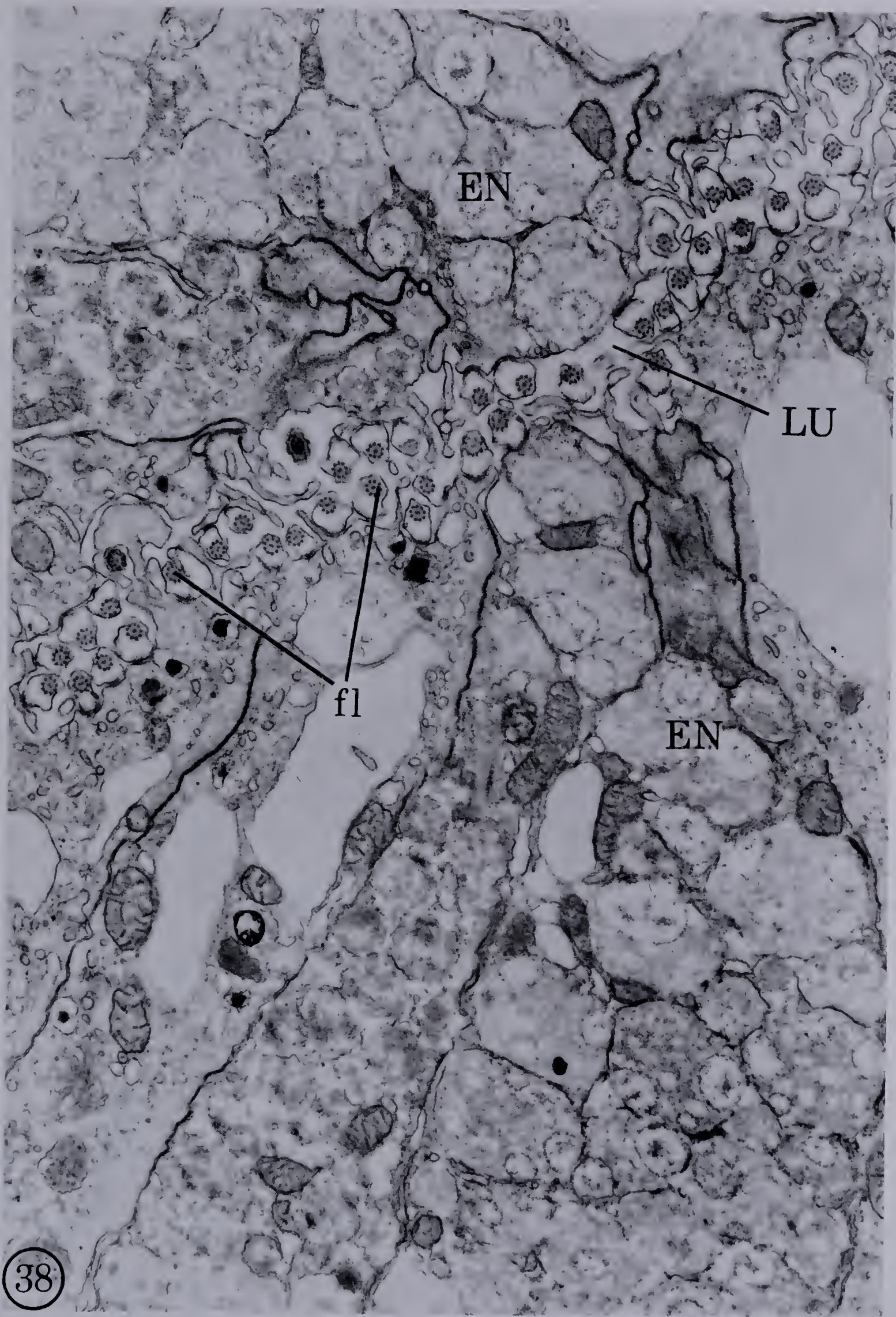


Fig. 38. A section through the lumen and part of the endoderm in the hypostome region, showing sections of flagella belonging to the gland cells, in the lumen.

EN, endoderm; fl, flagella; LU, lumen.

Osmium tetroxide fixation, Araldite embedding and uranyl acetate staining. X 16,000.



Figs. 39 and 40. A nerve cell with sensory hair (Fig. 39)

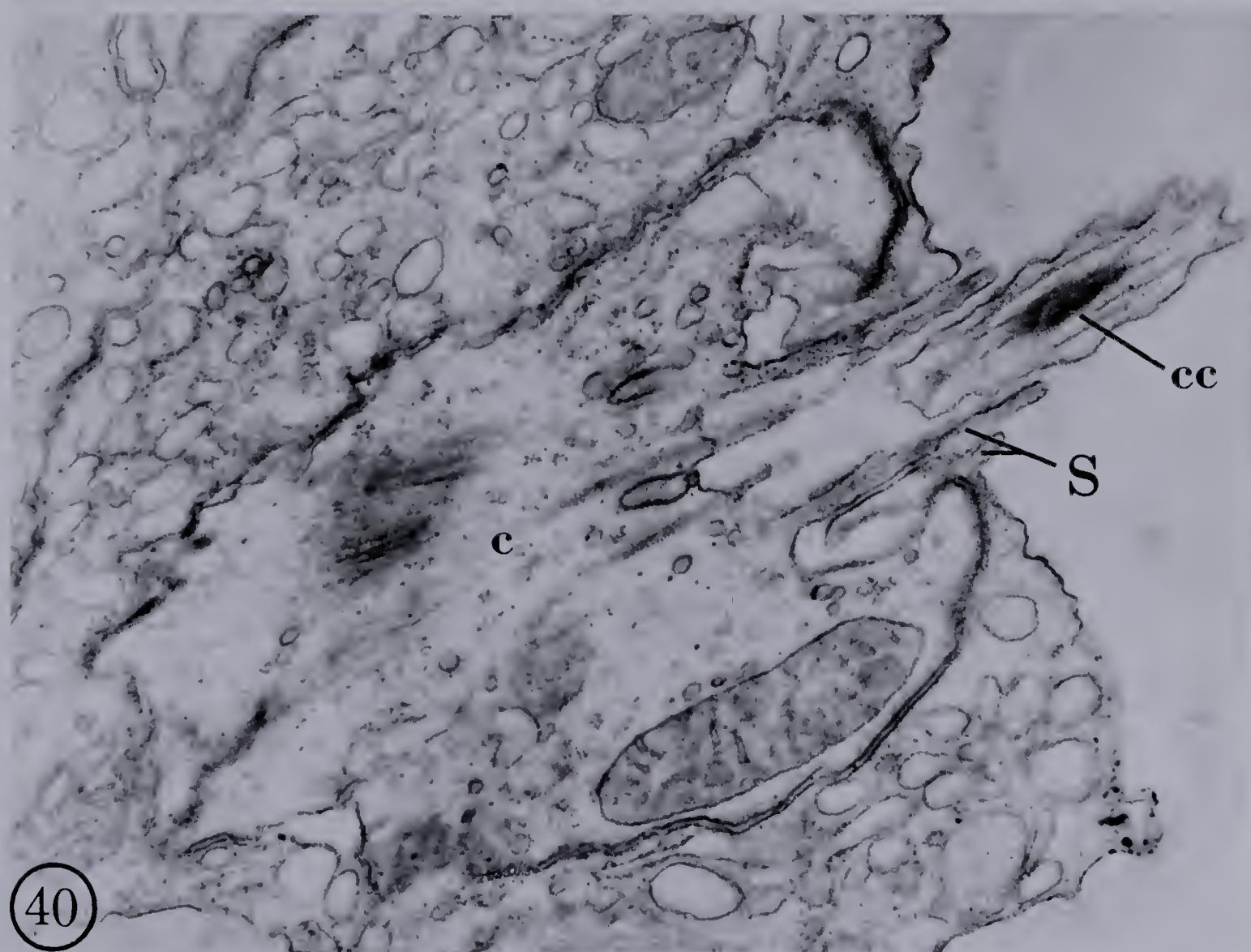
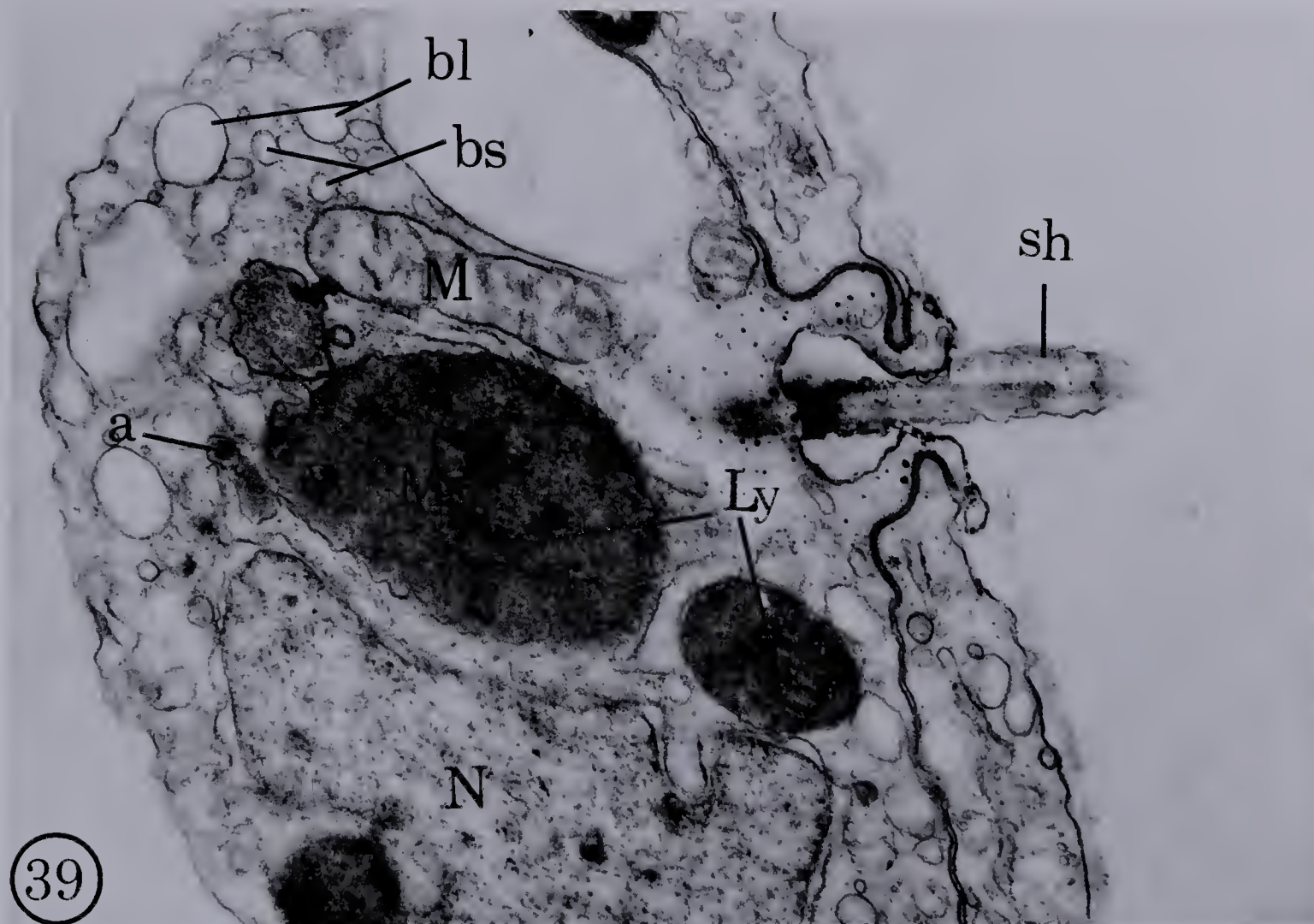
and a cnidocyte with cnidocil (Fig. 40) illustrating the difference in structure of the two. The nerve cell shows a multivesicular body, an A-type vesicle and the lysosomes, which have not been found in the cnidocytes. The cnidocil shows an electron-dense core and the supporting structures around it, which are missing at the base of the sensory hair.

a, A-type vesicle; bl, large B-type vesicles;
bs, small B-type vesicles; c, cnidocyte; cc, cnidocil;
Ly, lysosome; M, mitochondrion; Mv, multivesicular
body; N, nerve cell nucleus; S, supporting structures;
sh, sensory hair.

Osmium tetroxide fixation, Araldite embedding and
uranyl acetate staining.

Fig. 39 X 18,000.

Fig. 40 X 21,000.



Figs. 41 and 42. A nerve cell showing all the three types of vesicles (Fig. 41) compared with a cnidocyte (Fig. 42) which can be distinguished from the presence of the supporting structures around the base of the cnidocil.

a, A-type vesicle; bs, small B-type vesicle;
cv, C-type vesicles; c, cnidocyte; cc, cnidocil;
EC, ectoderm; Er, endoplasmic reticulum; G, Golgi complex; L, lipid body; ME, mesoglea; N, nerve cell nucleus; nu, nucleolus; OS, outer surface; P, pit; S, supporting structures; V, vacuole in epithelio-muscular cell.

Osmium tetroxide fixation, Araldite embedding and uranyl acetate staining.

Fig. 41 X 17,500.

Fig. 42 X 17,500.

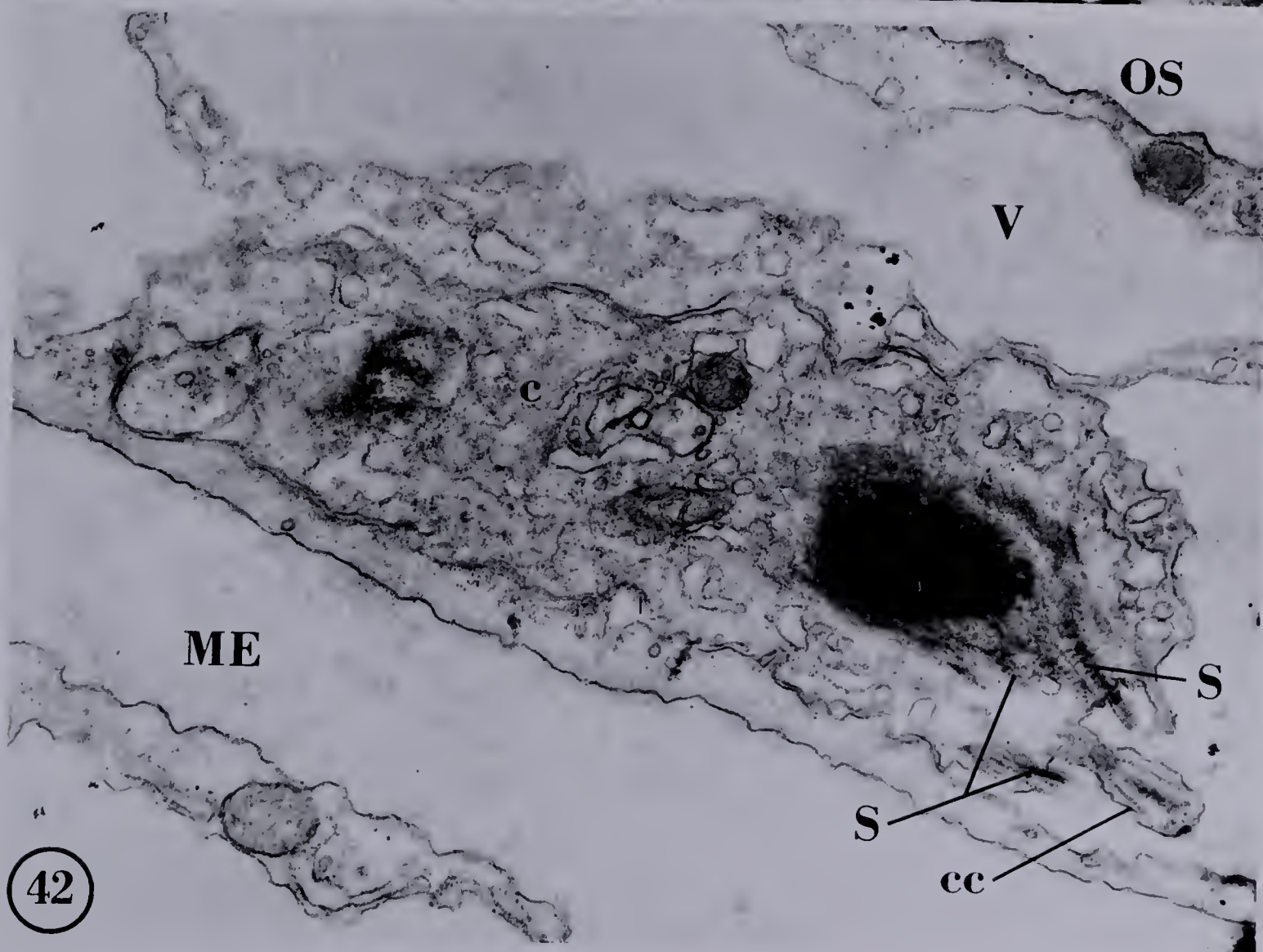
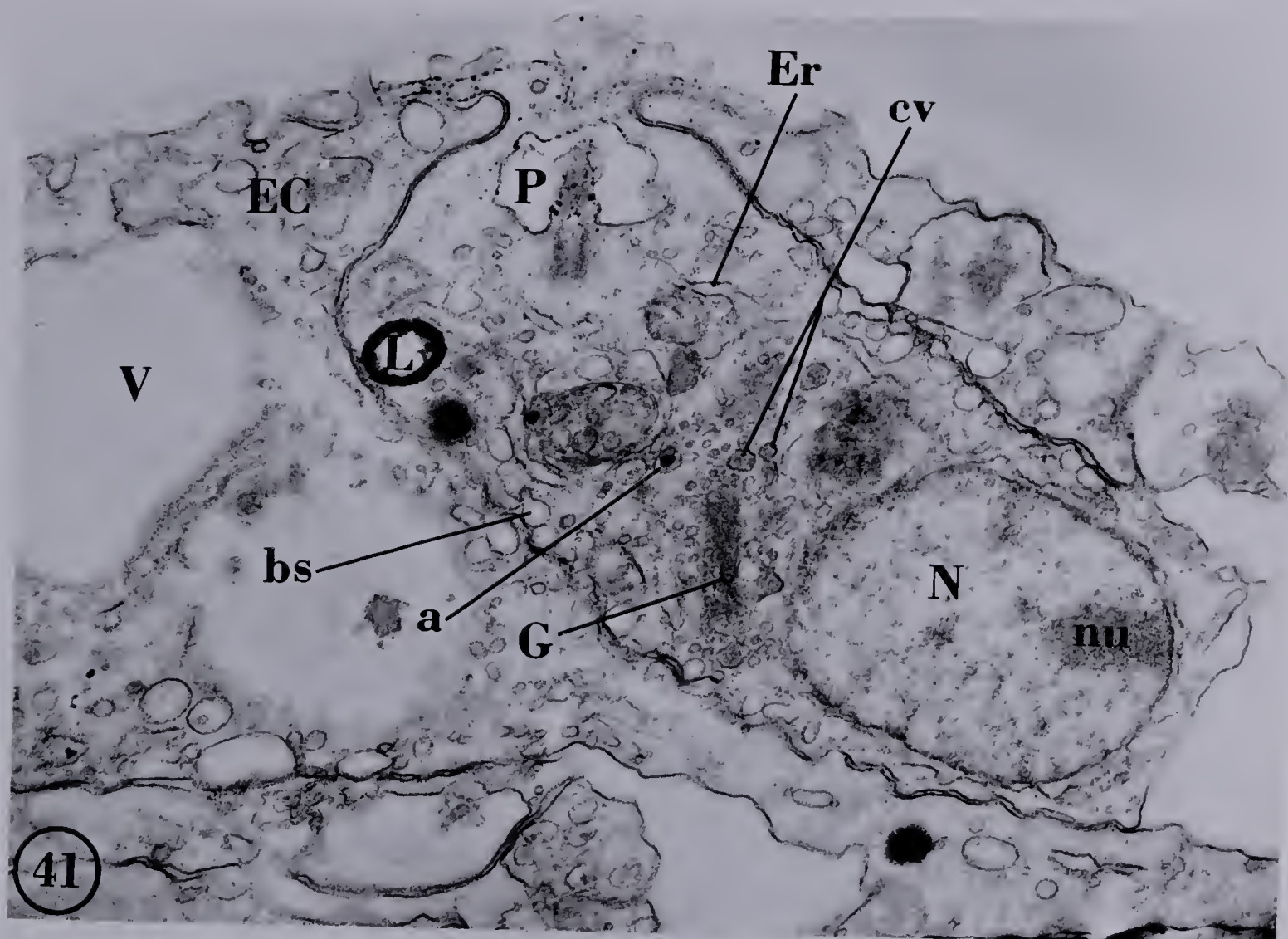


Fig. 43. One of the developing cnidoblasts, found in groups, in the section of the stalk at the base of the hydranth. Note the abundance of the endoplasmic reticulum in the cytoplasm and the cell membrane appears to be perforated at one point. X 18,000.

Er, endoplasmic reticulum; M, mitochondrion;

Nc, cnidoblast nucleus; nt, nematocyst.

Fig. 44. Longitudinal section of a cnidocil. X 31,500.

S, supporting structures; C, centriole.

Osmium tetroxide fixation, Araldite embedding and uranyl acetate staining.

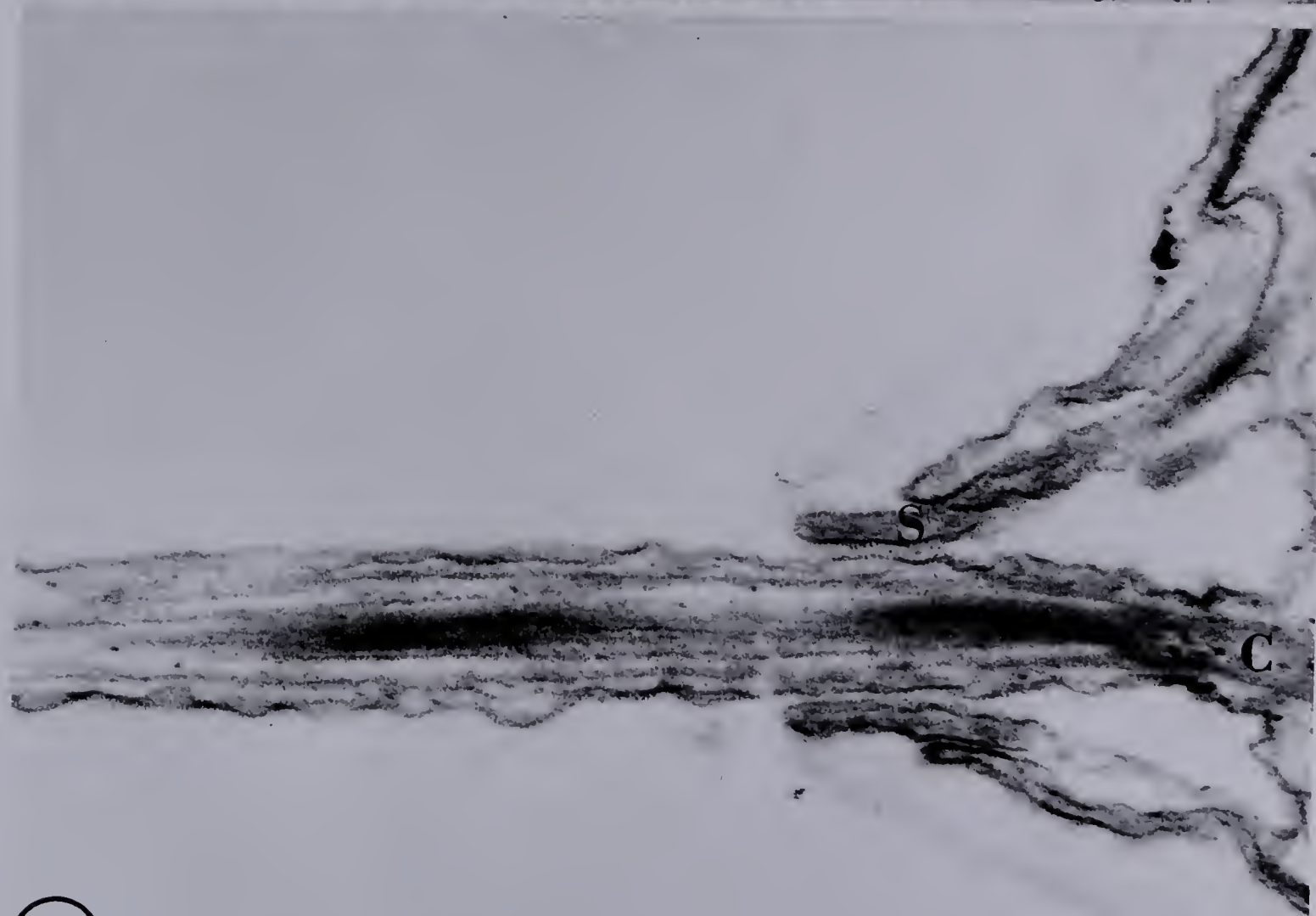
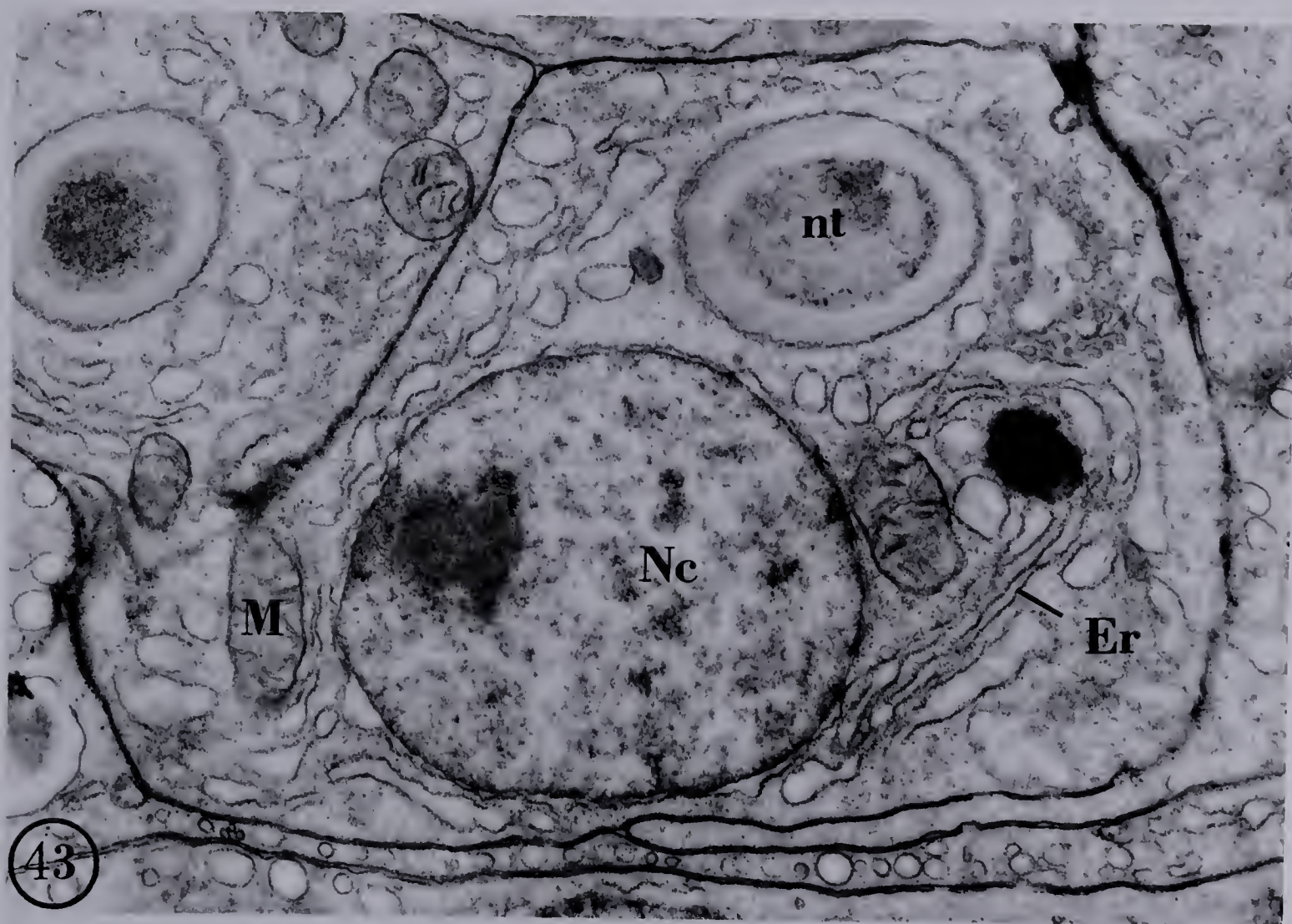


Fig. 45. Cross section of a cnidocil and the supporting structures in a silver stained preparation. Note the heavy deposition of silver on the surface of the cnidocil.

S, supporting structure; cc, cnidocil.

Picroformol fixation, silver stain, Araldite embedding and uranyl acetate counterstaining. X 78,000.

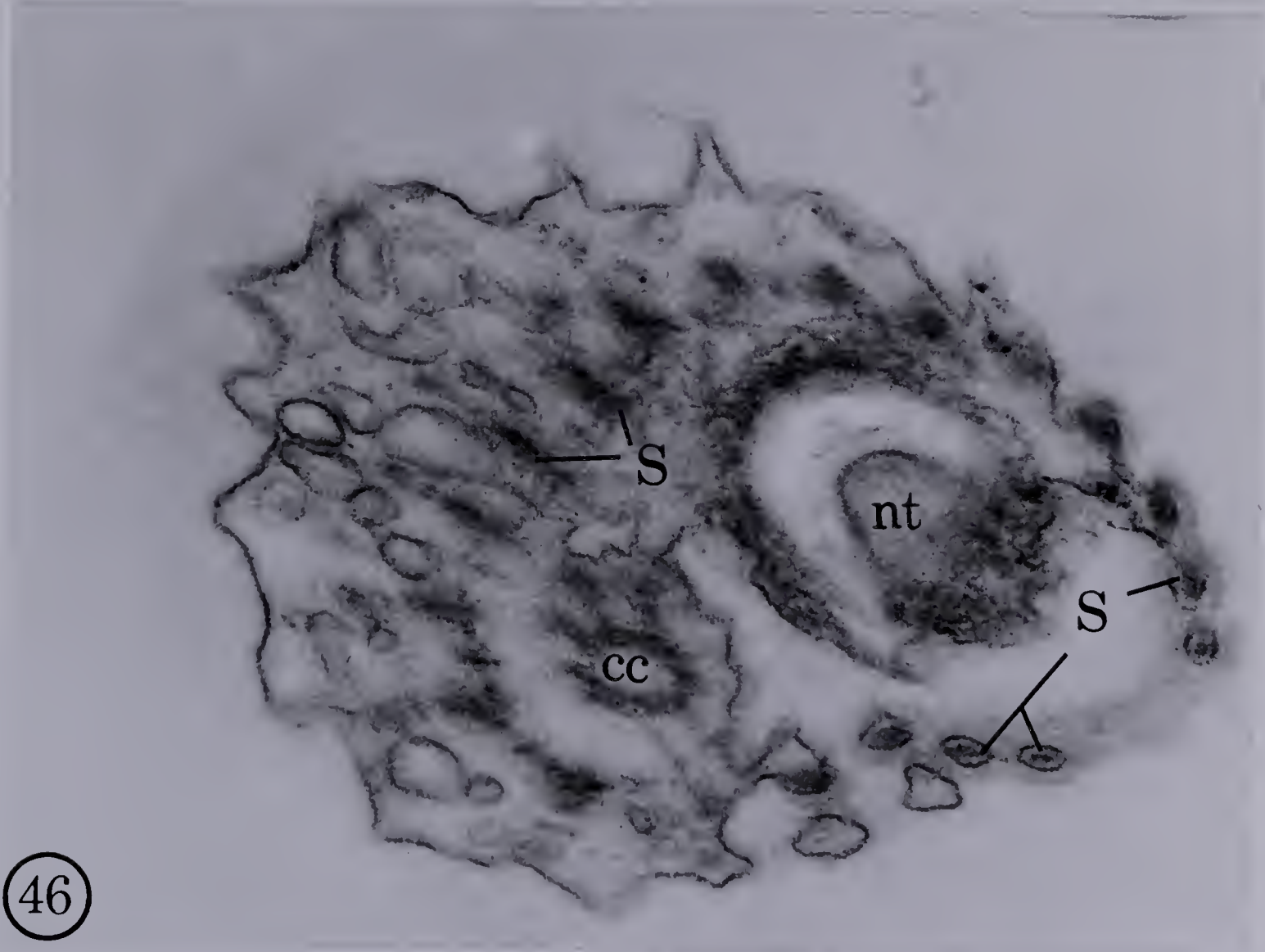
Fig. 46. Cross section of the cnidocyte in the region of the cnidocil.

cc, cnidocil; nt, apex of a nematocyst; S, supporting structures.

Osmium tetroxide fixation, Araldite embedding and uranyl acetate staining. X 40,000.



45



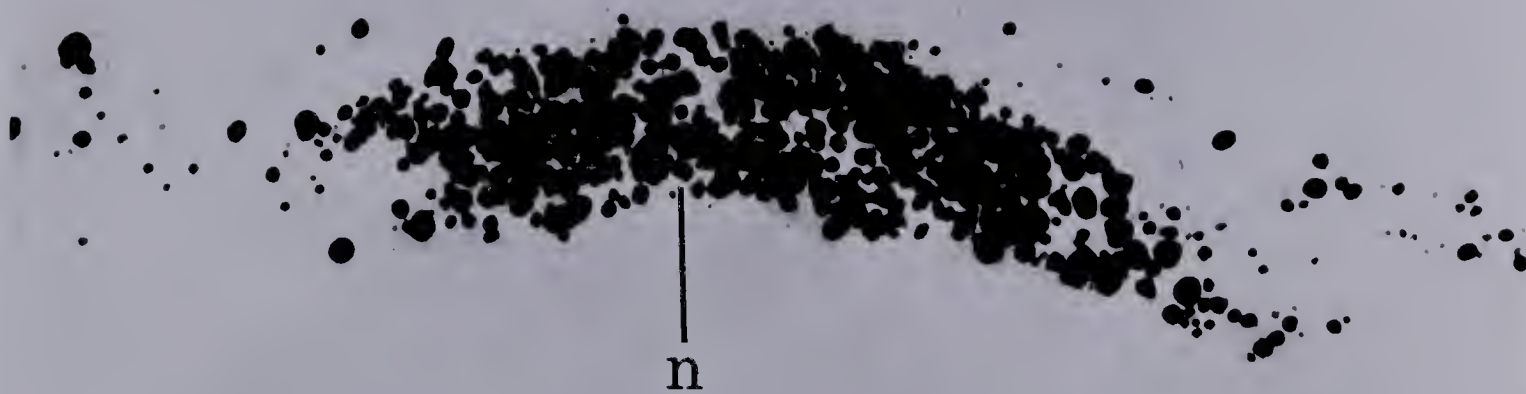
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Fig. 47. Section of a nerve process (n) in a silver-stained preparation. Note the heavy deposition of silver.
X 51,000.

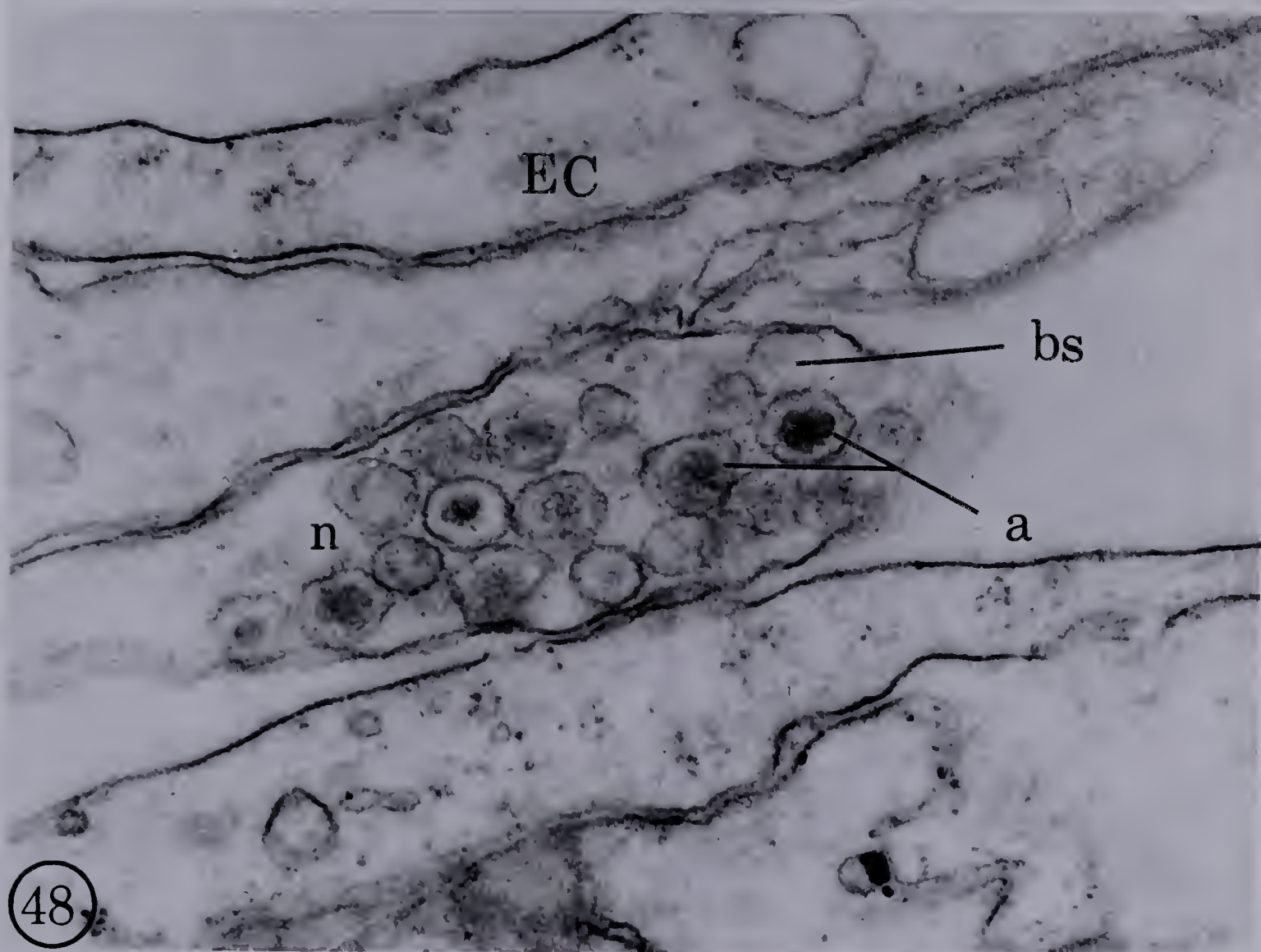
Fig. 48. Section of a nerve process (n) in an osmium-fixed preparation. Note the presence of two types of vesicles. X 74,000.

a, A-type vesicles; bs, small B-type vesicle;
EC, ectoderm.

Araldite embedding.



47



48

Fig. 49. A longitudinal section of the stem.

EC, ectoderm; c, cnidoblast; ME, mesoglea;
mp, muscle processes; ne, ectodermal nucleus;
V, vacuole.

Osmium tetroxide fixation, Araldite embedding
and uranyl acetate staining. X 11,000.

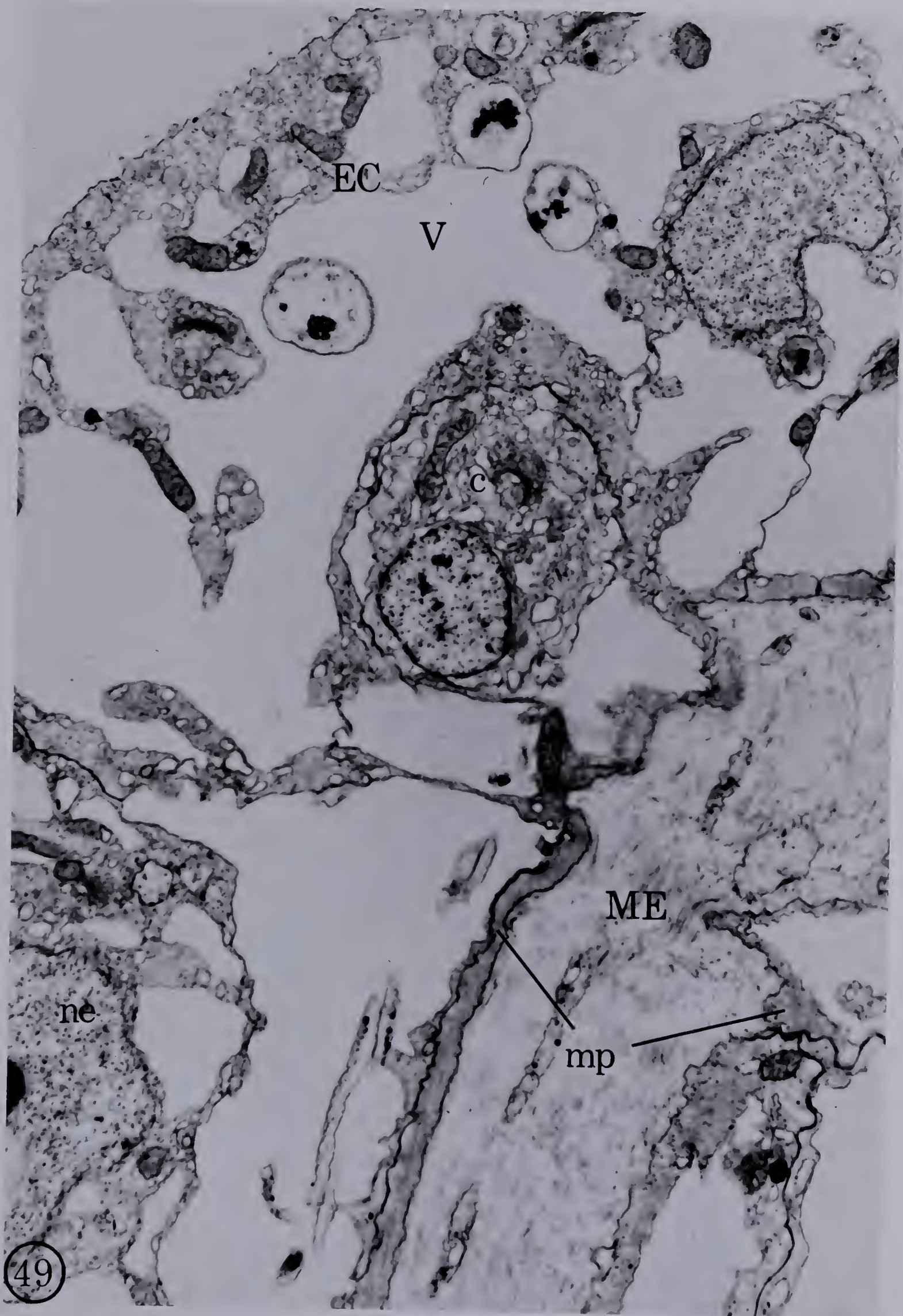
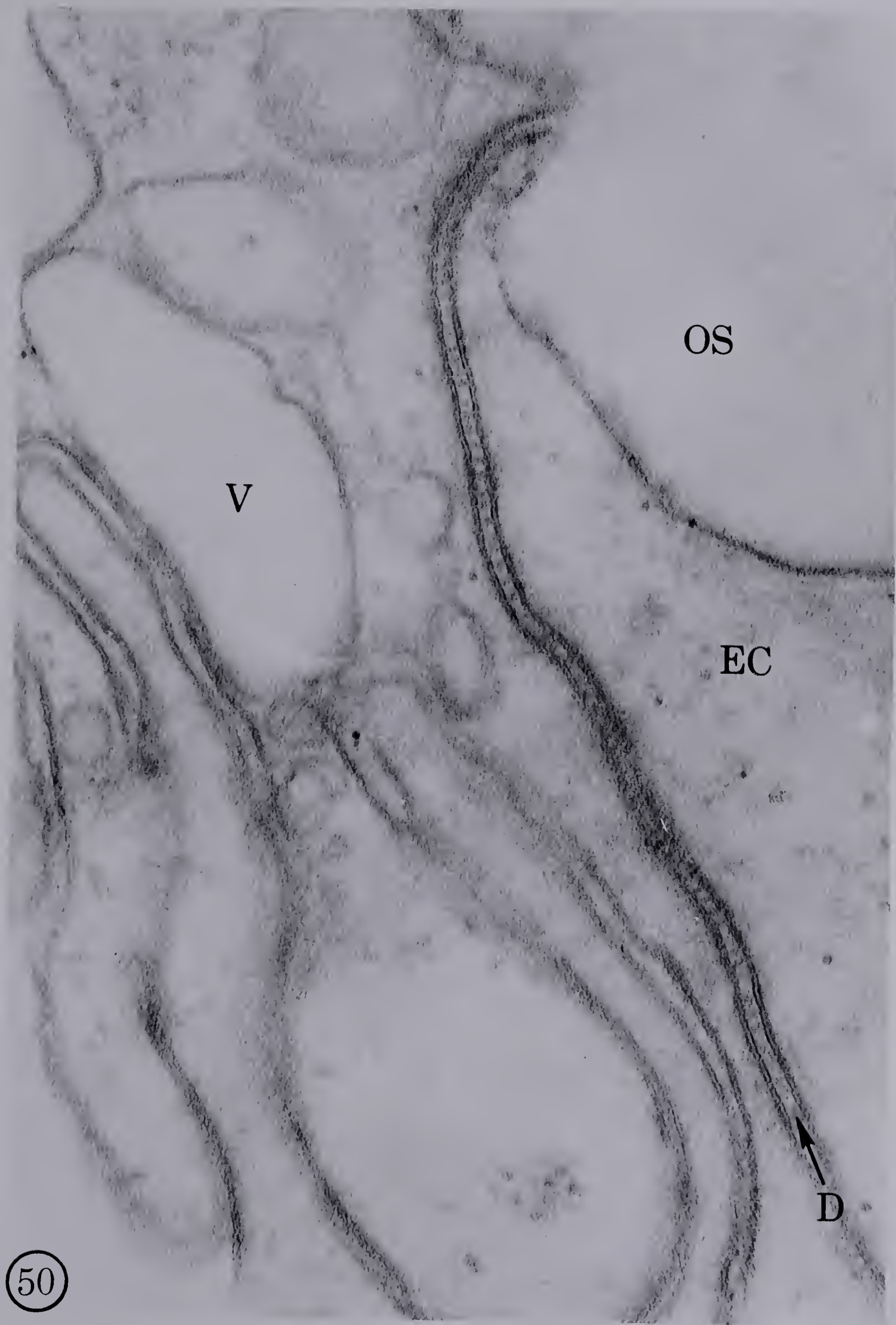


Fig. 50. Septate desmosomes between two epithelio-muscular cells similar to the one between a nerve cell and an epithelio-muscular cell seen in Fig. 32.

EC, ectoderm; D, desmosome; OS, outer surface;
V, vacuole.

Osmium tetroxide fixation, Araldite embedding and uranyl acetate staining. X 115,000.



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